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(54) Title: IRRIGATION SOLUTION CONTAINING MAPK INHIBITORS AND THEIR USE FOR TREATING PAIN AND INFLAMMATION

(57) Abstract

A method and solution for perioperatively inhibiting a variety of pain and inflammation processes at wounds from general surgical procedures including oral/dental procedures. The solution preferably includes at least one mitogen–activated protein kinase (MAPK) inhibitor and, optionally, additional multiple pain and inflammation inhibitory agents at dilute concentration in a physiologic carrier, such as saline or lactated Ringer's solution. The solution is applied by continuous irrigation of a wound during a surgical procedure for preemptive inhibition of pain and while avoiding undesirable side effects associated with oral, intramuscular, subcutaneous or intravenous application of larger doses of the agents. One preferred solution to inhibit pain and inflammation includes a MAPK inhibitor, a serotonin$_2$ antagonist, a serotonin$_3$ antagonist, a histamine antagonist, a serotonin agonist, a cyclooxygenase inhibitor, a neurokinin$_1$ antagonist, a neurokinin$_2$ antagonist, a purinoceptor antagonist, an ATP–sensitive potassium channel opener, a calcium channel antagonist, a bradykinin$_1$ antagonist, a bradykinin$_2$ antagonist and a μ–opioid agonist.

IRRIGATION SOLUTION CONTAINING MAPK INHIBITORS AND THEIR USE FOR TREATING PAIN AND INFLAMMATION

I. Field of the Invention

The present invention relates to surgical irrigation solutions and methods, and particularly for anti-inflammatory, anti-pain, anti-spasm and anti-restenosis surgical irrigation solutions.

II. Background of the Invention

Arthroscopy is a surgical procedure in which a camera, attached to a remote light source and video monitor, is inserted into an anatomic joint (e.g., knee, shoulder, etc.) through a small portal incision in the overlying skin and joint capsule. Through similar portal incisions, surgical instruments may be placed in the joint, their use guided by arthroscopic visualization. As arthroscopists' skills have improved, an increasing number of operative procedures, once performed by "open" surgical technique, now can be accomplished arthroscopically. Such procedures include, for example, partial meniscectomies and ligament reconstructions in the knee, shoulder acromioplasties and rotator cuff debridements and elbow synovectomies. As a result of widening surgical indications and the development of small diameter arthroscopes, wrist and ankle arthroscopies also have become routine.

Throughout each arthroscopy, physiologic irrigation fluid (e.g., normal saline or lactated Ringer's) is flushed continuously through the joint, distending the joint capsule and removing operative debris, thereby providing clearer intra-articular visualization. U.S. Patent 4,504,493 to Marshall discloses an isomolar solution of

For example, 5-HT applied to a human blister base (denuded skin) has been demonstrated to cause pain that can be inhibited by 5-HT_3 receptor antagonists. Richardson et al., (1985). Similarly, peripherally-applied bradykinin produces pain that can be blocked by bradykinin receptor antagonists. Sicuteri et al., 1965; Whalley et al., 1987; Dray, A., et. al., *Bradykinin and Inflammatory Pain*, Trends Neurosci. 16, pp. 99-104 (1993). Peripherally-applied histamine produces vasodilation, itching and pain which can be inhibited by histamine receptor antagonists. Rosenthal, 1977; Douglas, W.W., "Histamine and 5-Hydroxytryptamine (Serotonin) and their Antagonists", in Goodman, L.S., et. al., ed., *The Pharmacological Basis of Therapeutics*, MacMillan Publishing Company, New York, pp. 605-638 (1985); Rumore, M.M., et. al., *Analgesic Effects of Antihistaminics*, Life Sci 36, pp. 403-416 (1985). Combinations of these three agonists (5-HT, bradykinin and histamine) applied together have been demonstrated to display a synergistic pain-causing effect, producing a long-lasting and intense pain signal. Sicuteri et al., 1965; Richardson et al., 1985; Kessler, W., et. al., *Excitation of Cutaneous Afferent Nerve Endings In Vitro by a Combination of Inflammatory Mediators and Conditioning Effect of Substance P*, Exp. Brain Res. 91, pp. 467-476 (1992).

In the body, 5-HT is located in platelets and in central neurons, histamine is found in mast cells, and bradykinin is produced from a larger precursor molecule during tissue trauma, pH changes and temperature changes. Because 5-HT can be released in large amounts from platelets at sites of tissue injury, producing plasma levels 20-fold greater than resting levels (Ashton, J.H., et. al., *Serotonin as a Mediator of Cyclic Flow Variations in Stenosed Canine Coronary Arteries*, Circulation 73, pp. 572-578 (1986)), it is possible that endogenous 5-HT plays a role in producing postoperative pain, hyperalgesia and inflammation. In fact, activated platelets have been shown to excite peripheral nociceptors *in vitro*. Ringkamp, M., et. al., *Activated Human Platelets in Plasma Excite Nociceptors in Rat Skin, In Vitro*, Neurosci. Lett. 170, pp. 103-106 (1994). Similarly, histamine and bradykinin also are released into tissues during trauma. Kimura, E., et. al., *Changes in Bradykinin Level in Coronary Sinus Blood After the Experimental Occlusion of a Coronary Artery*, Am Heart J. 85, pp. 635-647 (1973); Douglas, 1985; Dray et. al. (1993).

In addition, prostaglandins also are known to cause pain and inflammation. Cyclooxygenase inhibitors, e.g., ibuprofen, are commonly used in non-surgical and post-operative settings to block the production of prostaglandins, thereby reducing prostaglandin-mediated pain and inflammation. Flower, R.J., et. al., *Analgesic-*

purported to be released. (2) Amitriptyline is known to be extensively metabolized by the liver. With oral administration, the concentration of amitriptyline in the operative site tissues may not have been sufficiently high for a long enough time period to inhibit the activity of postoperatively released 5-HT in the second study. (3) Since multiple inflammatory mediators exist, and studies have demonstrated synergism between the inflammatory mediators, blocking only one agent (5-HT) may not sufficiently inhibit the inflammatory response to tissue injury.

There have been a few studies demonstrating the ability of extremely high concentrations (1% - 3% solutions -- i.e., 10 - 30 mg per milliliter) of histamine$_1$ (H_1) receptor antagonists to act as local anesthetics for surgical procedures. This anesthetic effect is not believed to be mediated via H_1 receptors but, rather, due to a non-specific interaction with neuronal membrane sodium channels (similar to the action of lidocaine). Given the side effects (e.g., sedation) associated with these high "anesthetic" concentrations of histamine receptor antagonists, local administration of histamine receptor antagonists currently is not used in the perioperative setting.

III. Summary of the Invention

The present invention provides a solution comprising at least one mitogen-activated protein kinase (MAPK) inhibitor and, preferably, a mixture of multiple agents in low concentrations directed at inhibiting locally the mediators of pain, inflammation, spasm and restenosis in a physiologic electrolyte carrier fluid. The invention also provides a method for perioperative delivery of the irrigation solution containing these agents directly to a surgical site, where it works locally at the receptor and enzyme levels to preemptively limit pain, inflammation, spasm and restenosis at the site. Due to the local perioperative delivery method of the present invention, a desired therapeutic effect can be achieved with lower doses of agents than are necessary when employing other methods of delivery (i.e., intravenous, intramuscular, subcutaneous and oral). The anti-pain/anti-inflammation agents in the solution include, in addition to the at least one mitogen-activated protein kinase (MAPK) inhibitor(s), agents selected from the following classes of receptor antagonists and agonists and enzyme activators and inhibitors, each class acting through a differing molecular mechanism of action for pain and inflammation inhibition: (1) serotonin receptor antagonists; (2) serotonin receptor agonists; (3) histamine receptor antagonists; (4) bradykinin receptor antagonists; (5) kallikrein inhibitors; (6) tachykinin receptor antagonists, including neurokinin$_1$ and neurokinin$_2$

antagonists; (2) inhibitors of cell adhesion molecules, including (a) selectin inhibitors and (b) integrin inhibitors; (3) anti-chemotactic agents; (4) interleukin receptor antagonists (which also serve as anti-pain/anti-inflammation agents); and (5) intracellular signaling inhibitors including: (a) protein kinase C (PKC) inhibitors and protein tyrosine kinase inhibitors, (b) modulators of intracellular protein tyrosine phosphatases, (c) inhibitors of src homology$_2$ (SH2) domains, and (d) calcium channel antagonists. Such agents are useful in preventing restenosis of arteries treated by angioplasty, rotational atherectomy or other cardiovascular or general vascular therapeutic or diagnostic procedure.

The present invention also provides a method for manufacturing a medicament compounded as a dilute irrigation solution for use in continuously irrigating an operative site or wound during an operative procedure. The method entails dissolving in a physiologic electrolyte carrier fluid a plurality of anti-pain/anti-inflammatory agents, and for some applications anti-spasm agents and/or anti-restenosis agents, each agent included at a concentration of preferably no more than 100,000 nanomolar, and more preferably no more than 10,000 nanomolar.

The method of the present invention provides for the delivery of a dilute combination of multiple receptor antagonists and agonists and enzyme inhibitors and activators directly to a wound or operative site, during therapeutic or diagnostic procedures for the inhibition of pain, inflammation, spasm and restenosis. Since the active ingredients in the solution are being locally applied directly to the operative tissues in a continuous fashion, the drugs may be used efficaciously at extremely low doses relative to those doses required for therapeutic effect when the same drugs are delivered orally, intramuscularly, subcutaneously or intravenously. As used herein, the term "local" encompasses application of a drug in and around a wound or other operative site, and excludes oral, subcutaneous, intravenous and intramuscular administration. The term "continuous" as used herein encompasses uninterrupted application, repeated application at frequent intervals (e.g., repeated intravascular boluses at frequent intervals intraprocedurally), and applications which are uninterrupted except for brief cessations such as to permit the introduction of other drugs or agents or procedural equipment, such that a substantially constant predetermined concentration is maintained locally at the wound or operative site.

The advantages of low dose applications of agents are three-fold. The most important is the absence of systemic side effects that often limit the usefulness of these agents. Additionally, the agents selected for particular applications in the solutions of

accordance with the present invention before they can exert tissue damage, the benefit is more substantial than if given after the damage has been initiated.

Inhibiting more than one inflammatory, spasm or restenosis mediator by application of the multiple agent solution of the present invention has been shown to dramatically reduce the degree of inflammation, pain, and spasm, and theoretically should reduce restenosis. The irrigation solutions of the present invention include combinations of drugs, each solution acting on multiple receptors or enzymes. The drug agents are thus simultaneously effective against a combination of pathologic processes, including pain and inflammation, vasospasm, smooth muscle spasm and restenosis. The action of these agents is considered to be synergistic, in that the multiple receptor antagonists and inhibitory agonists of the present invention provide a disproportionately increased efficacy in combination relative to the efficacy of the individual agents. The synergistic action of several of the agents of the present invention are discussed, by way of example, below in the detailed descriptions of those agents.

In addition to arthroscopy, the solution of the present invention may also be applied locally to any human body cavity or passage, operative wound, traumatic wound (e.g., burns) or in any operative/interventional procedure in which irrigation can be performed. These procedures include, but are not limited to, urological procedures, cardiovascular and general vascular diagnostic and therapeutic procedures, endoscopic procedures and oral, dental and periodontal procedures. As used hereafter, the term "wound", unless otherwise specified, is intended to include surgical wounds, operative/interventional sites, traumatic wounds and burns.

Used perioperatively, the solution should result in a clinically significant decrease in operative site pain and inflammation relative to currently-used irrigation fluids, thereby decreasing the patient's postoperative analgesic (i.e., opiate) requirement and, where appropriate, allowing earlier patient mobilization of the operative site. No extra effort on the part of the surgeon and operating room personnel is required to use the present solution relative to conventional irrigation fluids.

IV. Brief Description of the Drawings

The present invention will now be described in greater detail, by way of example, with reference to the accompanying drawings in which:

FIGURE 6 provides a diagram of the G-Protein Coupled Receptor pathway including the signaling proteins responsible for ""crosstalk"" with the Growth Factor Receptor signaling pathway. Specific molecular sites of action for some drugs in a preferred urologic solution are identified.

FIGURE 7 provides a diagram of the G-Protein Coupled Receptor pathway. Specific molecular sites of action for some drugs in a preferred general surgical wound solution of the present invention are identified.

FIGURE 8 provides a diagram of the mechanism of action of nitric oxide (NO) donor drugs and NO causing relaxation of vascular smooth muscle. Physiologically, certain hormones and transmitters can activate a form of NO synthase in the endothelial cell through elevated intracellular calcium resulting in increased synthesis of NO. NO donors may generate NO extracellularly or be metabolized to NO within the smooth muscle cell. Extracellular NO can diffuse across the endothelial cell or directly enter the smooth muscle cell. The primary target of NO is the soluble guanylate cyclase (GC), leading to activation of a cGMP-dependent protein kinase (PKG) and subsequent extrusion of calcium from the smooth muscle cell via a membrane pump. NO also hyperpolarizes the cell by opening potassium channels which in turn cause closure of voltage-sensitive calcium channels. Thus, the synergistic interactions of calcium channel antagonists, potassium channel openers and NO donors are evident from the above signal transduction pathway.

FIGURES 9, 10A and 10B provide charts of the percent of vasoconstriction versus time in control arteries, in the proximal segment of subject arteries, and in the distal segment of subject arteries, respectively, for the animal study described in EXAMPLE VII herein demonstrating the effect on vasoconstriction of infusion with histamine and serotonin antagonists, used in the solutions of the present invention, during balloon angioplasty. FIGURES 11 and 12 provide charts of plasma extravasation versus dosage of amitriptyline, used in the solutions of the present invention, delivered intravenously and intra-articularly, respectively, to knee joints in which extravasation has been induced by introduction of 5-hydroxytryptamine in the animal study described in EXAMPLE VIII herein.

FIGURES 13, 14 and 15 provide charts of mean vasoconstriction (negative values) or vasodilation (positive values), ±1 standard error of the mean for the proximal (FIGURE 13), mid (FIGURE 14) and distal (FIGURE 15) segments of arteries treated with saline (N=4) or with a solution formulated in accordance with the

anti-restenosis agents include: (1) antiplatelet agents including: (a) thrombin inhibitors and receptor antagonists, (b) adenosine diphosphate (ADP) receptor antagonists (also known as purinoceptor$_1$ receptor antagonists), (c) thromboxane inhibitors and receptor antagonists and (d) platelet membrane glycoprotein receptor antagonists; (2) inhibitors of cell adhesion molecules, including (a) selectin inhibitors and (b) integrin inhibitors; (3) anti-chemotactic agents; (4) interleukin receptor antagonists (which also serve as anti-pain/anti-inflammation agents); and (5) intracellular signaling inhibitors including: (a) protein kinase C (PKC) inhibitors and protein tyrosine phosphatases, (b) modulators of intracellular protein tyrosine kinase inhibitors, (c) inhibitors of src homology$_2$ (SH2) domains, and (d) calcium channel antagonists. Such agents are useful in preventing restenosis of arteries treated by angioplasty, rotational atherectomy or other cardiovascular or general vascular therapeutic procedure.

In each of the surgical solutions of the present invention, the agents are included in low concentrations and are delivered locally in low doses relative to concentrations and doses required with conventional methods of drug administration to achieve the desired therapeutic effect. It is impossible to obtain an equivalent therapeutic effect by delivering similarly dosed agents via other (i.e., intravenous, subcutaneous, intramuscular or oral) routes of drug administration since drugs given systemically are subject to first- and second-pass metabolism. The concentration of each agent is determined in part based on its dissociation constant, K_d. As used herein, the term dissociation constant is intended to encompass both the equilibrium dissociation constant for its respective agonist-receptor or antagonist-receptor interaction and the equilibrium inhibitory constant for its respective activator-enzyme or inhibitor-enzyme interaction. Each agent is preferably included at a low concentration of 0.1 to 10,000 times K_d nanomolar, except for cyclooxygenase inhibitors, which may be required at larger concentrations depending on the particular inhibitor selected. Preferably, each agent is included at a concentration of 1.0 to 1,000 times K_d nanomolar and most preferably at approximately 100 times K_d nanomolar. These concentrations are adjusted as needed to account for dilution in the absence of metabolic transformation at the local delivery site. The exact agents selected for use in the solution, and the concentration of the agents, varies in accordance with the particular application, as described below.

A solution in accordance with the present invention can include just a single or multiple pain/inflammation inhibitory agent(s), a single or multiple anti-spasm

inflammatory, spasmodic and restenotic pathways is minimized by the surgical solutions. In these pathophysiologic pathways, the surgical solutions inhibit the cascade effect both "upstream" and "downstream".

An example of "upstream" inhibition is the cyclooxygenase antagonists in the setting of pain and inflammation. The cyclooxygenase enzymes (COX_1 and COX_2) catalyze the conversion of arachidonic acid to prostaglandin H which is an intermediate in the biosynthesis of inflammatory and nociceptive mediators including prostaglandins, leukotrienes, and thromboxanes. The cyclooxygenase inhibitors block "upstream" the formation of these inflammatory and nociceptive mediators. This strategy precludes the need to block the interactions of the seven described subtypes of prostanoid receptors with their natural ligands. A similar "upstream" inhibitor included in the surgical solutions is aprotinin, a kallikrein inhibitor. The enzyme kallikrein, a serine protease, cleaves the high molecular weight kininogens in plasma to produce bradykinins, important mediators of pain and inflammation. By inhibition of kallikrein, aprotinin effectively inhibits the synthesis of bradykinins, thereby providing an effective "upstream" inhibition of these inflammatory mediators.

The surgical solutions also make use of "downstream" inhibitors to control the pathophysiologic pathways. In vascular smooth muscle preparations that have been precontracted with a variety of neurotransmitters (e.g., serotonin, histamine, endothelin, and thromboxane) implicated in coronary vasospasm, ATP-sensitive potassium channel openers (KCOs) produce smooth muscle relaxation which is concentration dependent (Quast et al., 1994; Kashiwabara et al., 1994). The KCOs, therefore, provide a significant advantage to the surgical solutions in the settings of vasospasm and smooth muscle spasm by providing "downstream" antispasmodic effects that are independent of the physiologic combination of agonists initiating the spasmodic event (see FIGURES 2 and 4). Similarly, NO donors and voltage-gated calcium channel antagonists can limit vasospasm and smooth muscle spasm initiated by multiple mediators known to act earlier in the spasmodic pathway.

The following is a description of suitable drugs falling in the aforementioned classes of anti-inflammation/anti-pain agents, as well as suitable concentrations for use in solutions, of the present invention. While not wishing to be limited by theory, the justification behind the selection of the various classes of agents which is believed to render the agents operative is also set forth.

Isolated Coronary Arteries, Circulation 90, pp. 1141-53 (1994). Suitable serotonin$_{1B}$ receptor antagonists include yohimbine, N-[-methoxy-3-(4-methyl-1-piperanzinyl)phenyl]-2'-methyl-4'-(5-methyl-1, 2, 4-oxadiazol-3-yl)[1, 1-biphenyl]-4-carboxamide ("GR127935") and methiothepin. Therapeutic and preferred concentrations for use of these drugs in the solution of the present invention are set forth in Table 1.

B. Serotonin Receptor Agonists

5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors are known to inhibit adenylate cyclase activity. Thus including a low dose of these $serotonin_{1A}$, $serotonin_{1B}$ and $serotonin_{1D}$ receptor agonists in the solution should inhibit neurons mediating pain and inflammation. The same action is expected from $serotonin_{1E}$ and $serotonin_{1F}$ receptor agonists because these receptors also inhibit adenylate cyclase.

Buspirone is a suitable 1A receptor agonist for use in the present invention. Sumatriptan is a suitable 1A, 1B, 1D and 1F receptor agonist. A suitable 1B and 1D receptor agonist is dihydroergotamine. A suitable 1E receptor agonist is ergonovine. Therapeutic and preferred concentrations for these receptor agonists are provided in Table 2.

drug also has been shown to possess local anesthetic effects but the concentrations necessary for this effect are several orders higher than that necessary to block H_1 receptors, thus, the effects are believed to occur by different mechanisms. The histamine receptor antagonist concentration in the solution is sufficient to inhibit H_1 receptors involved in nociceptor activation, but not to achieve a "local anesthetic" effect, thereby eliminating the concern regarding systemic side effects.

Histamine receptors also are known to mediate vasomotor tone in the coronary arteries. *In vitro* studies in the human heart have demonstrated that the $histamine_1$ receptor subtype mediates contraction of coronary smooth muscle. Ginsburg, R., et al., *Histamine Provocation of Clinical Coronary Artery Spasm: Implications Concerning Pathogenesis of Variant Angina Pectoris*, American Heart J., Vol. 102, pp. 819-822, (1980). Some studies suggest that histamine-induced hypercontractility in the human coronary system is most pronounced in the proximal arteries in the setting of atherosclerosis and the associated denudation of the arterial endothelium. Keitoku, M. et al., *Different Histamine Actions in Proximal and Distal Human Coronary Arteries in Vitro*, Cardiovascular Research 24, pp. 614-622, (1990). Therefore, histamine receptor antagonists may be included in the cardiovascular irrigation solution.

Other suitable H_1 receptor antagonists include terfenadine, diphenhydramine, amitriptyline, mepyramine and tripolidine. Because amitriptyline is also effective as a $serotonin_2$ receptor antagonist, it has a dual function as used in the present invention. Suitable therapeutic and preferred concentrations for each of these H_1 receptor antagonists are set forth in Table 3.

is performed for both acute and chronic conditions, and thus an irrigation solution for arthroscopy could include both B_1 and B_2 receptor antagonists.

Suitable bradykinin receptor antagonists for use in the present invention include the following bradykinin$_1$ receptor antagonists: the [des-Arg^{10}] derivative of D-Arg-(Hyp^3-Thi^5-D-Tic^7-Oic^8)-BK ("the [des-Arg^{10}] derivative of HOE 140", available from Hoechst Pharmaceuticals); and [Leu^8] des-Arg^9-BK. Suitable bradykinin$_2$ receptor antagonists include: [D-Phe^7]-BK; D-Arg-(Hyp^3-Thi^{5,8}-D-Phe^7)-BK ("NPC 349"); D-Arg-(Hyp^3--D-Phe^7)-BK ("NPC 567"); and D-Arg-(Hyp^3-Thi^5-D-Tic^7-Oic^8)-BK ("HOE 140"). These compounds are more fully described in the previously incorporated Perkins et. al. 1993 and Dray et. al. 1993 references. Suitable therapeutic and preferred concentrations are provided in Table 4.

Table 4

Therapeutic and Preferred Concentrations of Pain/Inflammation Inhibitory Agents

Class of Agent	Therapeutic Concentrations (Nanomolar)	Preferred Concentrations (Nanomolar)
Bradykinin$_1$ Receptor Antagonists:		
[Leu^8] des-Arg^9-BK	1 - 1,000	50 - 500
[des-Arg^{10}] derivative of HOE 140	1 - 1,000	50 - 500
[leu^9] [des-Arg^{10}] kalliden	0.1 - 500	10 - 200
Bradykinin$_2$ Receptor Antagonists:		
[D-Phe^7]-BK	100 - 10,000	200 - 5,000
NPC 349	1 - 1,000	50 - 500
NPC 567	1 - 1,000	50 - 500
HOE 140	1 - 1,000	50 - 500

in the periphery after C-fiber activation, including vasodilation, plasma extravasation and degranulation of mast cells. Levine, J.D., et. al., *Peptides and the Primary Afferent Nociceptor*, J. Neurosci. 13, p. 2273 (1993). A suitable Substance P antagonist is ([D-Pro^9[spiro-gamma-lactam]Leu^{10},Trp^{11}]physalaemin-(1-11)) ("GR 82334"). Other suitable antagonists for use in the present invention which act on the NK_1 receptor are: 1-imino-2-(2-methoxy-phenyl)-ethyl)-7,7-diphenyl-4-perhydroisoindolone(3aR,7aR) ("RP 67580"); and 2S,3S-cis-3-(2-methoxybenzylamino)-2-benzhydrylquinuclidine ("CP 96,345"). Suitable concentrations for these agents are set forth in Table 6.

Table 6

Therapeutic and Preferred Concentrations of

Pain/Inflammation Inhibitory Agents

Class of Agent	Therapeutic Concentrations (Nanomolar)	Preferred Concentrations (Nanomolar)
Neurokinin$_1$ Receptor Subtype Antagonists		
GR 82334	1 - 1,000	10 - 500
CP 96,345	1-10,000	100-1,000
RP 67580	0.1-1,000	100-1,000

2. Neurokinin$_2$ Receptor Subtype Antagonists

Neurokinin A is a peptide which is colocalized in sensory neurons with substance P and which also promotes inflammation and pain. Neurokinin A activates the specific neurokinin receptor referred to as NK_2. Edmonds-Alt, S., et. al., *A Potent and Selective Non-Peptide Antagonist of the Neurokinin A (NK_2) Receptor*, Life Sci. 50:PL101 (1992). In the urinary tract, TKs are powerful spasmogens acting through only the NK_2 receptor in the human bladder, as well as the human urethra and ureter. Maggi, C.A., *Gen. Pharmacol.*, Vol. 22, pp. 1-24 (1991). Thus, the desired drugs for inclusion in a surgical solution for use in urological procedures would contain an antagonist to the NK_2 receptor to reduce spasm. Examples of suitable NK_2 antagonists include: ((S)-N-methyl-N-[4-(4-acetylamino-4-phenylpiperidino)-2- (3,4-dichlorophenyl)butyl]benzamide ("(±)-SR 48968"); Met-

Table 8

Therapeutic and Preferred Concentrations of

Pain/Inflammation Inhibitory Agents

Class of Agent	Therapeutic Concentrations (Nanomolar)	Preferred Concentrations (Nanomolar)
CGRP Receptor Antagonist:		
I-CGRP-(8-37)	1-1,000	10-500

H. Interleukin Receptor Antagonist

Interleukins are a family of peptides, classified as cytokines, produced by leukocytes and other cells in response to inflammatory mediators. Interleukins (IL) may be potent hyperalgesic agents peripherally. Ferriera, S.H., et. al., *Interleukin-1β as a Potent Hyperalgesic Agent Antagonized by a Tripeptide Analogue*, Nature 334, p. 698 (1988). An example of a suitable IL-1β receptor antagonist is Lys-D-Pro-Thr, which is a truncated version of IL-1β. This tripeptide inhibits the activation of IL-1β receptors. Suitable concentrations for this agent are provided in Table 9.

Table 9

Therapeutic and Preferred Concentrations of

Pain/Inflammation Inhibitory Agents

Class of Agent	Therapeutic Concentrations (Nanomolar)	Preferred Concentrations (Nanomolar)
Interleukin Receptor Antagonist:		
Lys-D-Pro-Thr	1-1,000	10-500

W.N., et. al., eds.), p. 258 (1989). The molecular targets for these drugs are type I and type II cyclooxygenases (COX-1 and COX-2). These enzymes are also known as Prostaglandin H Synthase (PGHS)-1 (constitutive) and -2 (inducible), and catalyze the conversion of arachidonic acid to Prostaglandin H which is an intermediate in the biosynthesis of prostaglandins and thromboxanes. The COX-2 enzyme has been identified in endothelial cells, macrophages, and fibroblasts. This enzyme is induced by IL-1 and endotoxin, and its expression is upregulated at sites of inflammation. Constitutive activity of COX-1 and induced activity of COX-2 both lead to synthesis of prostaglandins which contribute to pain and inflammation.

NSAIDs currently on the market (diclofenac, naproxen, indomethacin, ibuprofen, etc.) are generally nonselective inhibitors of both isoforms of COX, but may show greater selectively for COX-1 over COX-2, although this ratio varies for the different compounds. Use of COX-1 and 2 inhibitors to block formation of prostaglandins represents a better therapeutic strategy than attempting to block interactions of the natural ligands with the seven described subtypes of prostanoid receptors. Reported antagonists of the eicosanoid receptors (EP-1, EP-2, EP-3) are quite rare and only specific, high affinity antagonists of the thromboxane A2 receptor have been reported. Wallace, J. and Cirino, G. *Trends in Pharm. Sci.*, Vol. 15 pp. 405-406 (1994).

The oral, intravenous or intramuscular use of cyclooxygenase inhibitors is contraindicated in patients with ulcer disease, gastritis or renal impairment. In the United States, the only available injectable form of this class of drugs is ketorolac (Toradol™), available from Syntex Pharmaceuticals, which is conventionally used intramuscularly or intravenously in postoperative patients but, again, is contraindicated for the above-mentioned categories of patients. The use of ketorolac, or any other cyclooxygenase inhibitor(s), in the solution in substantially lower dosages than currently used perioperatively may allow the use of this drug in otherwise contraindicated patients. The addition of a cyclooxygenase inhibitor to the solutions of the present invention adds a distinct mechanism for inhibiting the production of pain and inflammation during arthroscopy or other therapeutic or diagnostic procedure.

Preferred cyclooxygenase inhibitors for use in the present invention are keterolac and indomethacin. Of these two agents, indomethacin is less preferred because of the relatively high dosages required. Therapeutic and preferred concentrations for use in the solution are provided in Table 11.

carboxylic acid, 2-acetylhydrazide ("SC 19220"). A suitable thromboxane receptor subtype antagonist is [15-[1α, 2β(5Z), 3β, 4α]-7-[3-[2-(phenylamino)-carbonyl] hydrazino] methyl]-7-oxobicyclo-[2,2,1]-hept-2-yl]-5-heptanoic acid ("SQ 29548"). Suitable concentrations for these agents are set forth in Table 13.

Table 13

Therapeutic and Preferred Concentrations of

Pain/Inflammation Inhibitory Agents

Class of Agent	Therapeutic Concentrations (Nanomolar)	Preferred Concentrations (Nanomolar)
Eicosanoid EP-1 Antagonist:		
SC 19220	100-10,000	500-5,000

K. Leukotriene Receptor Antagonists

The leukotrienes (LTB_4, LTC_4, and LTD_4) are products of the 5-lipooxygenase pathway of arachidonic acid metabolism that are generated enzymatically and have important biological properties. Leukotrienes are implicated in a number of pathological conditions including inflammation. Specific antagonists are currently being sought by many pharmaceutical companies for potential therapeutic intervention in these pathologies. Halushka, P.V., et al., Annu. Rev. Pharmacol. Toxicol. 29: 213-239 (1989); Ford-Hutchinson, A. Crit. Rev. Immunol. 10: 1-12 (1990). The LTB_4 receptor is found in certain immune cells including eosinophils and neutrophils. LTB_4 binding to these receptors results in chemotaxis and lysosomal enzyme release thereby contributing to the process of inflammation. The signal transduction process associated with activation of the LTB_4 receptor involves G-protein-mediated stimulation of phosphotidylinositol (PI) metabolism and elevation of intracellular calcium (see FIGURE 2).

An example of a suitable leukotriene B_4 receptor antagonist is SC (+)-(S)-7-(3-(2-(cyclopropylmethyl)-3-methoxy-4-[(methylamino)-carbonyl]phenoxy(propoxy)-3,4-dihydro-8-propyl-2H-1-benzopyran-2-propanoic acid ("SC 53228"). Concentrations for this agent that are suitable for the practice of the present invention are provided in Table 14. Other suitable leukotriene B_4 receptor antagonists include [3-[-2(7-chloro-2-quinolinyl)ethenyl]phenyl] [[3-(dimethylamino-3-oxopropyl)thio]

Table 15

Therapeutic and Preferred Concentrations of

Pain/Inflammation Inhibitory Agents

Class of Agent	Therapeutic Concentrations (Nanomolar)	Preferred Concentrations (Nanomolar)
T-Opioid Agonist:		
DAMGO	0.1-100	0.5-20
sufentanyl	0.01-50	1-20
fentanyl	0.1-500	10-200
PL 017	0.05-50	0.25-10
δ-Opioid Agonist:		
DPDPE	0.1-500	1.0-100
κ-Opioid Agonist:		
U50,488	0.1-500	1.0-100

M. Purinoceptor Antagonists and Agonists

Extracellular ATP acts as a signaling molecule through interactions with P_2 purinoceptors. One major class of purinoceptors are the P_{2x} purinoceptors which are ligand-gated ion channels possessing intrinsic ion channels permeable to Na^+, K^+, and Ca^{2+}. P_{2x} receptors described in sensory neurons are important for primary afferent neurotransmission and nociception. ATP is known to depolarize sensory neurons and plays a role in nociceptor activation since ATP released from damaged cells stimulates P_{2X} receptors leading to depolarization of nociceptive nerve-fiber terminals. The $P2X_3$ receptor has a highly restricted distribution (Chen, C.C., et. al., Nature, Vol. 377, pp. 428-431 (1995)) since it is selectively expressed in sensory C-fiber nerves that run into the spinal cord and many of these C-fibers are known to carry the receptors for painful stimuli. Thus, the highly restricted localization of expression for

neuronal receptors and thus will inhibit effects due to nerve stimulation and release of inflammatory mediators. Quast, U., et. al., *Cellular Pharmacology of Potassium Channel Openers in Vascular Smooth Muscle,* Cardiovasc. Res., Vol. 28, pp. 805-810 (1994).

Synergistic interactions between endothelin (ET_A) antagonists and openers of ATP-sensitive potassium channels (KCOs) are expected in achieving vasorelaxation or smooth muscle relaxation. A rationale for dual use is based upon the fact that these drugs have different molecular mechanisms of action in promoting relaxation of smooth muscle and prevention of vasospasm. An initial intracellular calcium elevation in smooth muscle cells induced by the ET_A receptor subsequently triggers activation of voltage-dependent channels and the entry of extracellular calcium which is required for contraction. Antagonists of the ET_A receptor will specifically block this receptor mediated effect but not block increases in calcium triggered by activation of other G-protein coupled receptors on the muscle cell.

Potassium-channel opener drugs, such as pinacidil, will open these channels causing K^+ efflux and hyperpolarization of the cell membrane. This hyperpolarization will act to reduce contraction mediated by other receptors by the following mechanisms: (1) it will induce a reduction in intracellular free calcium through inhibition of voltage-dependent Ca^{2+} channels by reducing the probability of opening L-type or T-type calcium channels, (2) it will restrain agonist induced (receptor operated channels) Ca^{2+} release from intracellular sources through inhibition of inositol triphosphate (IP_3) formation, and (3) it will lower the efficiency of calcium as an activator of contractile proteins. Consequently, combined actions of these two classes of drugs will clamp the target cells into a relaxed state or one which is more resistant to activation.

Suitable ATP-sensitive K^+ channel openers for the practice of the present invention include: (-)pinacidil; cromakalim; nicorandil; minoxidil; N-cyano-N'-[1,1-dimethyl-[2,2,3,3-^{3}H]propyl]-N"-(3-pyridinyl)guanidine ("P 1075"); and N-cyano-N'-(2-nitroxyethyl)-3-pyridinecarboximidamide monomethansulphonate ("KRN 2391"). Concentrations for these agents are set forth in Table 17.

synthase (NOS) catalyzes the conversion of L-arginine to NO which acts as a diffusible second messenger and mediates responses in adjacent smooth muscle cells (see FIGURE 8). NO is continuously formed and released by the vascular endothelium under basal conditions which inhibits contractions and controls basal coronary tone and is produced in the endothelium in response to various agonists (such as acetylcholine) and other endothelium dependent vasodilators. Thus, regulation of NO synthase activity and the resultant levels of NO are key molecular targets controlling vascular tone (see FIGURE 8). Muramatsu, K., et. al., Coron. Artery Dis., Vol. 5, pp. 815-820 (1994).

Synergistic interactions between NO donors and openers of ATP-sensitive potassium channels (KCOs) are expected to achieve vasorelaxation or smooth muscle relaxation. A rationale for dual use is based upon the fact that these drugs have different molecular mechanisms of action in promoting relaxation of smooth muscle and prevention of vasospasm. There is evidence from cultured coronary arterial smooth muscle cells that the vasoconstrictors: vasopressin, angotensin II and endothelin, all inhibit K_{ATP} currents through inhibition of protein kinase A. In addition, it has been reported that K_{ATP} current in bladder smooth muscle is inhibited by muscarinic agonists. The actions of NO in mediating smooth muscle relaxation occur via independent molecular pathways (described above) involving protein kinase G (see FIGURE 8). This suggests that the combination of the two classes of agents will be more efficacious in relaxing smooth muscle than employing a single class of agent alone.

Suitable nitric oxide donors for the practice of the present invention include nitroglycerin, sodium nitroprusside, the drug FK 409, FR 144420, 3-morpholinosydnonimine , or linsidomine chlorohydrate, ("SIN-1"); and S-nitroso-N-acetylpenicillamine ("SNAP"). Concentrations for these agents are set forth in Table 18.

Suitable endothelin receptor antagonists include: cyclo(D-Asp-Pro-D-Val-Leu-D-Trp) ("BQ 123"); (N,N-hexamethylene)-carbamoyl-Leu-D-Trp-(CHO)-D-Trp-OH ("BQ 610"); (R)2-([R-2-[(s)-2-([1-hexahydro-1H-azepinyl]-carbonyl]amino-4-methyl-pentanoyl) amino-3-(3[1-methyl-1H-indodyl])propionylamino-3(2-pyridyl) propionic acid ("FR 139317"); cyclo(D-Asp-Pro-D-Ile-Leu-D-Trp) ("JKC 301"); cyclo(D-Ser-Pro-D-Val-Leu-D-Trp) ("JK 302"); 5-(dimethylamino)-N-(3,4-dimethyl-5-isoxazolyl)-1-naphthalenesulphonamide ("BMS 182874"); and N-[1-Formyl-N-[N-[(hexahydro-1H-azepin-1-yl)carbonyl]-L-leucyl]-D-tryptophyl]-D-tryptophan ("BQ 610"). Concentrations for a representative three of these agents is set forth in Table 19.

Table 19

Therapeutic and Preferred Concentrations of

Spasm Inhibitory Agents

Class of Agent	Therapeutic Concentrations (Nanomolar)	Preferred Concentrations (Nanomolar)
Endothelin Receptor Antagonists:		
BQ 123	0.01-1,000	10-1,000
FR 139317	1-100,000	100-10,000
BQ 610	0.01 to 10,000	10 - 1,000

4. Ca^{2+} Channel Antagonists

Calcium channel antagonists are a distinct group of drugs that interfere with the transmembrane flux of calcium ions required for activation of cellular responses mediating neuroinflammation. Calcium entry into platelets and white blood cells is a key event mediating activation of responses in these cells. Furthermore, the role of bradykinin receptors and neurokinin receptors (NK_1 and NK_2) in mediating the neuroinflammation signal transduction pathway includes increases in intracellular calcium, thus leading to activation of calcium channels on the plasma membrane. In many tissues, calcium channel antagonists, such as nifedipine, can reduce the release of arachidonic acid, prostaglandins, and leukotrienes that are evoked by various

ET_A receptor in coronary arterial smooth muscle, and hence speculated that ET-1 is an endogenous agonist of voltage-sensitive calcium channels. It has been found that the sustained phase of intracellular calcium elevation in smooth muscle cells induced by ET_A receptor activation requires extracellular calcium and is at least partially blocked by nicardipine. Thus, the inclusion of a calcium channel antagonist would be expected to synergistically enhance the actions of an ET_A antagonist when combined in a surgical solution.

Calcium channel antagonists and ATP-sensitive potassium channel openers likewise exhibit synergistic action. Potassium channels that are ATP-sensitive (K_{ATP}) couple the membrane potential of a cell to the cell's metabolic state via sensitivity to adenosine nucleotides. K_{ATP} channels are inhibited by intracellular ATP but are stimulated by intracellular nucleotide diphosphates. The activity of these channels is controlled by the electrochemical driving force to potassium and intracellular signals (e.g., ATP or a G-protein), but are not gated by the membrane potential per se. K_{ATP} channels hyperpolarize the membrane and thus allow them to control the resting potential of the cell. ATP-sensitive potassium currents have been discovered in skeletal muscle, brain, and vascular and nonvascular smooth muscle. Binding studies with radiolabeled ligands have confirmed the existence of ATP-sensitive potassium channels which are the receptor targets for the potassium-channel opener drugs such as pinacidil. Opening of these channels causes potassium efflux and hyperpolarizes the cell membrane. This hyperpolarization (1) induces a reduction in intracellular free calcium through inhibition of voltage-dependent Ca^{2+} channels by reducing the probability of opening L-type or T-type calcium channels, (2) restrains agonist induced (at receptor operated channels) Ca^{2+} release from intracellular sources through inhibition of inositol triphosphate (IP_3) formation, and (3) lowers the efficiency of calcium as an activator of contractile proteins. The combined actions of these two classes of drugs (ATP-sensitive potassium channel openers and calcium channel antagonists) will clamp the target cells into a relaxed state or one which is more resistant to activation.

Finally, calcium channel antagonists and tachykinin and bradykinin antagonists exhibit synergistic effects in mediating neuroinflammation. The role of neurokinin receptors in mediating neuroinflammation has been established. The neurokinin$_1$ (NK_1) and neurokinin$_2$ (NK_2) receptor (members of the G-protein coupled superfamily) signal transduction pathway includes increases in intracellular calcium, thus leading to activation of calcium channels on the plasma membrane. Similarly,

and still more preferably also with anti-pain/anti-inflammation agents in the solutions of the present invention.

1. Antiplatelet Agents

At sites of arterial injury, platelets adhere to collagen and fibrinogen via specific cell surface receptors, and are then activated by several independent mediators. A variety of agonists are able to activate platelets, including collagen, ADP, thromboxane A2, epinephrine and thrombin. Collagen and thrombin serve as primary activators at sites of vascular injury, while ADP and thromboxane A2 act to recruit additional platelets into a growing platelet plug. The activated platelets degranulate and release other agents which serve as chemoattractants and vasoconstrictors, thus promoting vasospasm and platelet accumulation. Thus, anti-platelet agents can be antagonists drawn from any of the above agonist-receptor targets.

Since platelets play such an important role in the coagulation cascade, oral antiplatelet agents have been routinely administered to patients undergoing vascular procedures. Indeed, because of this multiplicity of activators and observations that single antiplatelet agents are not effective, some investigators have concluded that a combined treatment protocol is necessary for effectiveness. Recently, Willerson and coworkers reported the intravenous use of 3 combined agents, ridogrel (an antagonist of thromboxane A2), ketanserin (a serotonin antagonist) and clopidogrel (an ADP antagonist). They found that the combination of 3 antagonists inhibited several relevant platelet functions and reduced neointimal proliferation in a canine coronary angioplasty model (JACC Abstracts, Feb. 1995). It is still uncertain which approach to treatment of coronary thrombosis will be most successful. One possibility would be to include an antiplatelet agent and an antithrombotic agent in the cardiovascular and general vascular solutions of the present invention.

a. Thrombin Inhibitors and Receptor Antagonists

Thrombin plays a central role in vascular lesion formation and is considered the principal mediator of thrombogenesis. Thus, thrombus formation at vascular lesion sites during and after PTCA (percutaneous transluminal coronary angioplasty) or other vascular procedure is central to acute reocclusion and chronic restenosis. This process can be interrupted by application of direct anti-thrombins, including hirudin and its synthetic peptide analogs, as well as thrombin receptor antagonist peptides (Harker, et al., 1995, Am. J. Cardiol 75, 12B). Thrombin is also a potent

Table 21

Therapeutic and Preferred Concentrations of

Restenosis Inhibitory Agents

Class of Agent	Therapeutic/Preferred Concentrations (Nanomolar)	More preferred (Nanomolar)
Thrombin Inhibitors and Receptor Antagonists:		
hirudin	0.00003-3/0.0003-0.3	0.03
hirulog	0.2-20,000/2-2,000	200

b. ADP Receptor Antagonists (Purinoceptor Antagonists)

Ticlopidine, an analog of ADP, inhibits both thromboxane and ADP-induced platelet aggregation. It is likely that ticlopidine blocks interaction of ADP with its receptor, thereby inhibiting signal transduction by this G-protein coupled receptor on the surface of platelet membranes. A preliminary study showed it to be more effective than aspirin in combination with dipyridamole. However, the clinical use of ticlopidine has been limited because it causes neutropenia. Clopidogrel, a ticlopidine analog, is thought to have fewer adverse side effects than ticlopidine and is currently being studied for prevention of ischemic events. It is theorized that these agents may be suitable for use in the solutions of the present invention.

c. Thromboxane Inhibitors and Receptor Antagonists

Agents currently utilized for conventional methods of treatment of thrombosis rely upon aspirin, heparin and plasminogen activators. Aspirin irreversibly acetylates cyclooxygenase and inhibits the synthesis of thromboxane A2 and prostacyclin. While data support a benefit of aspirin for PTCA, the underlying efficacy of aspirin is considered as only partial or modest. This is likely due to platelet activation through thromboxane A2 independent pathways that are not blocked by aspirin induced acetylation of cyclooxygenase. Platelet aggregation and thrombosis may occur despite aspirin treatment. Aspirin in combination with dipyridamole has also been shown to reduce the incidence of acute complication during PTCA but not the incidence of restenosis.

A 0.25 mg/kg bolus of c7E3 followed by 10 μg/min intravenous infusion for 12 hrs produced greater than 80% blockade of GPIIb/IIIa receptors for the duration of the infusion. This was correlated with a greater than 80% inhibition of platelet aggregation. The antibody was coadministered with heparin and an increased risk of bleeding was noted. Additional information was obtained from the EPIC trial which showed a significant reduction in the primary end point, a composite of death rate, incidence of nonfatal myocardial infarction and need for coronary revascularization, and suggested a long term benefit. Tcheng, (1995) Am. Heart J. 130, 673-679. A phase IV study (EPILOG) designed to address safety and efficacy issues with c7E3 Fab is planned or in progress. This monoclonal antibody can also be classified as a platelet membrane glycoprotein receptor antagonist directed against the glycoprotein IIb/IIIa receptor.

The platelet glycoprotein IIb/IIIa receptor blocker, integrelin, is a cyclic heptapeptide that is highly specific for this molecular target. In contrast to the antibody, it has a short biologic half-life (about 10 minutes). The safety and efficacy of integrelin was first evaluated in the Phase II Impact trial. Either 4 or 12 hour intravenous infusions of 1.0 μg/kg/min of integrelin were utilized (Topol, E., 1995 Am. J. Cardiol, 27B-33B). It was provided in combination with other agents (heparin, aspirin) and was shown to exhibit potent anti-platelet aggregation properties (>80%). A phase III study, the IMPACT II trial, of 4000 patients showed that integrelin markedly reduced ischemic events in patients who had undergone Rotablator atherectomy (JACC Abstracts, 1996). Suitable concentrations of the drugs c7E3 and integrelin for use in the present invention are set forth below.

In addition, two peptidomimetics, MK-383 (Merck) and RO 4483 (Hoffmann-LaRoche), have been studied in Phase II clinicals. Since these are both small molecules, they have a short half-life and high potency. However, these seem to also have less specificity, interacting with other closely related integrins. It is theorized that these peptidomimetics may also be suitable for use in the present invention.

i. Protein Kinase C (PKC) Inhibitors

Protein kinase C (PKC) plays a crucial role in cell-surface signal transduction for a number of physiological processes. PKC isozymes can be activated as downstream targets resulting from initial activation of either G-protein coupled receptors (e.g., serotonin, endothelin, etc.) or growth-factor receptors such as PDGF. Both of these receptor classes play important roles in mediating vascular spasm and restenosis subsequent to coronary balloon angioplasty procedures.

Molecular cloning analysis has revealed that PKC exists as a large family consisting of at least 8 subspecies (isozymes). These isozymes differ substantially in structure and mechanism for linking receptor activation to changes in the proliferative response of specific cells. Expression of specific isozymes is found in a wide variety of cell types, including: platelets, neutrophils, myeloid cells, and smooth muscle cells. Inhibitors of PKC are therefore likely to effect signaling pathways in several cell types unless the inhibitor shows isozyme specificity. Thus, inhibitors of PKC can be predicted to be effective in blocking the proliferative response of smooth muscle cells and may also have an anti-inflammatory effect in blocking neutrophil activation and subsequent attachment. Several inhibitors have been described and initial reports indicate an IC_{50} of 50 nM for calphostin C inhibitory activity. G-6203 (also known as Go 6976) is a new, potent PKC inhibitor with high selectivity for certain PKC isotypes with IC_{50} values in the 2-10 nM range. Concentrations of these and another drug, GF 109203X, also known as Go 6850 or bisindoylmaleimide I (available from Warner-Lambert), that are believed to be suitable for use in the present invention are set forth below.

sequence of intracellular events leading to enhanced proliferation and neointimal thickening. An inhibitor of PDGF kinase activity would be expected to prevent proliferation and enhance the probability of success following cardiovascular and general vascular procedures. Any of several related tyrphostin compounds have potential as specific inhibitors of PDGF-receptor tyrosine kinase activity (IC_{50}s *in vitro* in the 0.5-1.0 μM range), since they have little effect on other protein kinases and other signal transduction systems. To date, only a few of the many tyrphostin compounds are commercially available, and suitable concentrations for these agents as used in the present invention are set forth below. In addition, staurosporine has been reported to demonstrate potent inhibitory effects against several protein tyrosine kinases of the src subfamily and a suitable concentration for this agent as used in the present invention also is set forth below.

Table 24

Therapeutic and Preferred Concentrations of

Restenosis Inhibitory Agents

Class of Agent	Therapeutic/Preferred Concentrations (Nanomolar)	More preferred (Nanomolar)
Protein Kinase Inhibitors		
lavendustin A	10-100,000/100-10,000	10,000
tyrphostin AG1296	10-100,000/100-20,000	10,000
tyrphostin AG1295	10-100,000/100-20,000	10,000
staurosporine	1-100,000/10-10,000	1,000

iii. MAP Kinase Inhibitors

The mitogen-activated protein (MAP) kinases are a group of protein serine/threonine kinases that are activated in response to a variety of extracellular stimuli and function in transducing signals from the cell surface to the nucleus. The MAP kinase cascade is one of the major signaling pathways that transmit signals from growth factors, hormones and inflammatory cytokines to intermediate early genes. In

The role of p38 mitogen-activated protein kinase (MAPK) in biochemical inflammatory responses of human fibroblasts and vascular endothelial cells to IL-1 was investigated by use of SB203580, which specifically inhibits the enzyme. Actions of IL-1 that are selectively controlled by p38 MAPK are the regulation of prostaglandin H synthase-2 (also known as COX-2), metalloproteinases, and IL-6 at different levels. (Ridley SH et al. (1997) J. Immunol. 158:3165-73). SB203580 inhibited (50% inhibitory concentration approximately 0.5 μM) IL-1-induced phosphorylation of hsp 27 (an indicator of p38 MAPK activity) in fibroblasts without affecting the other known IL-1-activated protein kinase pathways (p42/p44 MAPK, p54 MAPK/c-Jun N-terminal kinase). In addition, SB203580 significantly inhibited IL-1-stimulated IL-6, (30 to 50% at 1 μM) but not IL-8 production from human fibroblasts (gingival and dermal) and umbilical vein endothelial cells. IL-1 induction of steady state level of IL-6 mRNA was not significantly inhibited, which is consistent with p38 MAPK regulating IL-6 production at the translational level. Importantly, SB203580 strongly inhibited IL-1-stimulated prostaglandin production by fibroblasts and human umbilical vein endothelial cells. This was associated with the inhibition of the induction of COX-2 protein and mRNA. Since many cell types associated with inflammation, such as monocytes, endothelial cells and fibroblasts (including synovial) express the COX-2 gene at high levels upon activation by cytokines and extracellular stimuli, the MAPK inhibitor is expected to exhibit anti-inflammatory activity against all of these cellular types.

MAPK inhibitors may also be effective as cartilage protective agents when applied locally to tissues of the joint in a variety of inflammatory or pathophysiological conditions. SB203580 was found to inhibit the stimulation of collagenase-1 and stromelysin-1 production by IL-1 without affecting synthesis of tissue inhibitor metalloproteinases (TIMP)-1. Furthermore, SB203580 prevented the increase in collagenase-1 and stromelysin-1 mRNA stimulated by IL-1. In a model of cartilage breakdown, short-term IL-1-stimulated proteoglycan resorption and inhibition of proteoglycan synthesis were unaffected by SB 203580, while longer term collagen breakdown was prevented.

p38 MAP kinase is involved in tumor necrosis factor (TNF)-induced cytokine expression and drugs which function as inhibitors of p38 MAP kinase activity block the production of proinflammatory cytokines, as described below (Beyaert, R. et al., EMBO J. 1996 15:1914-23). TNF treatment of cells activated the p38 MAPK pathway, as revealed by increased phosphorylation of p38 MAPK itself, activation of

downstream effects on many cell types in the joint (synovial fibroblasts and chondrocytes) thus inhibiting subsequent pathological effects such as infiltration of inflammatory cells into the joint, synovial hyperplasia, synovial cell activation, cartilage breakdown and inhibition of cartilage matrix synthesis. Thus, a MAPK inhibitor should block the propagation of the inflammatory response by the aforementioned cytokines, and thereby interrupt the disease process.

From the molecular and cellular mechanism of action defined for MAP kinase inhibitors, such as SB203580, these compounds are expected to exhibit anti-inflammatory action when applied intraoperatively in an irrigation solution directly to a tissue or a joint. In particular, MAPK inhibitors are expected to be effective drugs delivered by an irrigation solution during an arthroscopic, urologic, or general surgical procedure (periprocedurally). The MAPK inhibitor may be delivered alone, or in combination with other small molecule drugs, peptides, proteins, recombinant chimeric proteins, antibodies, or gene therapy vectors (viral and nonviral) to the spaces of the joint, urogenital tract, or any cavity of the body. For example, the MAPK inhibitor can exert its actions on any cells associated with the fluid spaces of the joint and structures comprising the joint and are involved in the normal function of the joint or are present due to a pathological condition. These cells and structures include, but are not limited to: synovial cells including both Type A fibroblast and type B macrophage cells; the cartilaginous components of the joint such as chondrocytes; cells associated with bone, including periosteal cells, osteoblasts, osteoclasts; the immunological components such as inflammatory cells including lymphocytes, mast cells, monocytes, eosinophils; and other cells like fibroblasts; and combinations of the above.

The MAPK inhibitor may be delivered in a formulation useful for introduction and administration of the drug into the targeted tissue or joint that would enhance the delivery, uptake, stability or pharmacokinetics of the inhibitor drug. The formulation could include, but is not limited to, administration using microparticles, microspheres or nanoparticles composed of lipids, proteins, carbohydrates, synthetic organic compounds, or inorganic compounds. Examples of formulation molecules include, but are not limited to, lipids capable of forming liposomes or other ordered lipid structures, cationic lipids, hydrophilic polymers, polycations (e.g. protamine, spermidine, polylysine), peptide or synthetic ligands and antibodies capable of targeting materials to specific cell types, gels, slow release matrices, soluble and insoluble particles, as well as formulation elements not listed.

with other anti-pain, anti-inflammatory, anti-spasm, and/or anti-restenotic agents to inhibit restenosis. For example, a MAPK inhibitor could be included in Example VII. Representative examples of MAPK inhibitor compounds suitable for the invention include, for example, 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4- pyridyl)-1H-imidazole (SB203580), 4-(3-Iodophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole (SB203580-iodo), 4-(4-fluorophenyl)-2-(4-hydroxyphenyl)-5-(4- pyridyl)-1H-imidazole (SB202190), 4-(4-fluorophenyl)-2-(4-nitrophenyl)-5-(4-pyridyl)-1H-imidazole (PD 169316), and 2'-amino-3'-methoxyflavone (PD98059). Representative useful dosages for these compounds are listed in the Table below.

MAP Kinase Inhibitors

Compounds	Therapeutic Preferred Concentrations (nM)	Most Preferred Concentrations (nM)
SB 203580	0.5-50,000	50-10,000
SB 203580 iodo	0.5-50,000	50-10,000
SB 202190	0.2-20,000	20-5,000
PD 98059	0.1-10,000	10-2,000
PD 169316	1-100,000	10-20,000

b. Modulators of Intracellular Protein Tyrosine Phosphatases.

Non-transmembrane protein tyrosine phosphatases (PTPases) containing src-homology$_2$ SH2 domains are known and nomenclature refers to them as SH-PTP1 and SH-PTP2. In addition, SH-PTP1 is also known as PTP1C, HCP or SHP. SH-PTP2 is also known as PTP1D or PTP2C. Similarly, SH-PTP1 is expressed at high levels in hematopoietic cells of all lineages and all stages of differentiation, and the SH-PTP1 gene has been identified as responsible for the motheaten (me) mouse phenotype and this provides a basis for predicting the effects of inhibitors that would block its interaction with its cellular substrates. Stimulation of neutrophils with chemotactic peptides is known to result in the activation of tyrosine kinases that mediate neutrophil responses (Cui, et al., 1994 J. Immunol.) and PTPase activity modulates agonist induced activity by reversing the effects of tyrosine kinases

in blocking proliferation and the restenosis process after PTCA or other vascular procedure. One RTK target of current interest is the PDGF receptor.

At least 20 cytosolic proteins have been identified that contain SH2 domains and function in intracellular signaling. The distribution of SH2 domains is not restricted to a particular protein family, but found in several classes of proteins, protein kinases, lipid kinases, protein phosphatases, phospholipases, Ras-controlling proteins and some transcription factors. Many of the SH2-containing proteins have known enzymatic activities while others (Grb2 and Crk) function as "linkers" and "adapters" between cell surface receptors and "downstream" effector molecules (Marengere, L., et al., Nature 369:502-505, 1994). Examples of proteins containing SH2 domains with enzymatic activities that are activated in signal transduction include, but are not limited to, the src subfamily of protein tyrosine kinases (src ($pp60^{c\text{-}src}$), abl, lck, fyn, fgr and others), phospholipaseCγ (PLCγ), phosphatidylinositol 3-kinase (PI-3-kinase), p21-ras GTPase activating protein (GAP) and SH2 containing protein tyrosine phosphatases (SH-PTPases) (Songyang, et al., Cell 72, 767-778, 1993). Due to the central role these various SH2-proteins occupy in transmitting signals from activated cell surface receptors into a cascade of additional molecular interactions that ultimately define cellular responses, inhibitors which block specific SH2 protein binding are desirable as agents for a variety of potential therapeutic applications.

In addition, the regulation of many immune/inflammatory responses is mediated through receptors that transmit signals through non-receptor tyrosine kinases containing SH2 domains. T-cell activation via the antigen specific T-cell receptor (TCR) initiates a signal transduction cascade leading to lymphokine secretion and T-cell proliferation. One of the earliest biochemical responses following TCR activation is an increase in tyrosine kinase activity. In particular, neutrophil activation is in part controlled through responses of the cell surface immunoglobulin G receptors. Activation of these receptors mediates activation of unidentified tyrosine kinases which are known to possess SH2 domains. Additional evidence indicates that several src-family kinases (lck, blk, fyn) participate in signal transduction pathways leading from cytokine and integrin receptors and hence may serve to integrate stimuli received from several independent receptor structures. Thus, inhibitors of specific SH2 domains have the potential to block many neutrophil functions and serve as anti-inflammatory mediators.

a. "Crosstalk" and Convergence in Major Signaling Pathways

The molecular switches responsible for cell signaling have been traditionally divided into two major discrete signaling pathways, each comprising a distinct set of protein families that act as transducers for a particular set of extracellular stimuli and mediating distinct cell responses. One such pathway transduces signals from neurotransmitters and hormones through G-protein coupled receptors (GPCRs) to produce contractile responses using intracellular targets of trimeric G proteins and Ca^{2+} (see FIGURE 2). These stimuli and their respective receptors mediate smooth muscle contraction and may induce vasospasm in the context of PTCA or other cardiovascular or general vascular therapeutic or diagnostic procedure. Examples of signaling molecules involved in mediating spasm through the GPCR pathway are 5-HT and endothelin for which antagonists have been included acting via their respective G-protein coupled receptors.

A second major pathway transduces signals from growth factors, such as PDGF, through tyrosine kinases, adaptor proteins and the Ras protein into regulation of cell proliferation and differentiation (see FIGURES 2 and 5). This pathway may also be activated during PTCA or other cardiovascular or general vascular procedure leading to a high incidence of vascular smooth muscle cell proliferation. An example of a restenosis drug target is the PDGF-receptor.

Signals transmitted from neurotransmitters and hormones stimulate either of two classes of receptors: G-protein-coupled receptors, composed of seven-helix transmembrane regions, or ligand-gated ion channels. "Downstream" signals from both kinds of receptors converge on controlling the concentration of cytoplasmic Ca^{2+} which triggers contraction in smooth muscle cells (see FIGURE 2). Each GPCR transmembrane receptor activates a specific class of trimeric G proteins, including G_q, G_i or many others. G_I and/or $G_{\beta K}$ subunits activate phospholipase C_β, resulting in activation of protein kinase C (PKC) and an increase in the levels of cytoplasmic calcium by release of calcium from intracellular stores.

Growth factor signaling, such as mediated by PDGF, converges on regulation of cell growth. This pathway depends upon phosphorylation of tyrosine residues in receptor tyrosine kinases and "downstream" enzymes (phospholipase C_K, discussed above with regard to tyrosine kinases). Activation of the PDGF-receptor also leads to stimulation of PKC and elevation of intracellular calcium, common steps shared by the GPCRs (see FIGURE 2). It is now recognized that ligand-independent "crosstalk"

sustained phase of intracellular calcium elevation in smooth muscle cells induced by ET_A receptor activation requires extracellular calcium and is at least partially blocked by nicardipine. Since activation of both 5-HT_2 receptors and ET_A receptors is mediated through calcium, the inclusion of a PKC inhibitor is expected to synergistically enhance the actions of antagonists to both of these receptors when combined in a surgical solution (see FIGURES 2 and 4).

d. Synergistic Effects of Protein Tyrosine Kinase Inhibitors and Calcium Channel Antagonists

The mitogenic effect of PDGF (or basic fibroblast growth factor or insulin-like-growth-factor-1) is mediated through receptors that possess intrinsic protein tyrosine kinase activity. The substrates for PDGF phosphorylation are many and lead to activation of mitogen-activated protein kinases (MAPK) and ultimately proliferation (see FIGURE 5). The endothelin, 5-HT and thrombin receptors, which are members of the G-protein coupled superfamily, trigger a signal transduction pathway which includes increases in intracellular calcium, leading to activation of calcium channels on the plasma membrane. Thus, calcium channel antagonists interfere with a common mechanism employed by these GPCRs. It has recently been shown that activation of certain GPCRs, including endothelin and bradykinin, leads to a rapid increase in tyrosine phosphorylation of a number of intracellular proteins. Some of the proteins phosphorylated parallel those known necessary for mitogenic stimulation. The rapidity of the process was such that changes were detectable in seconds and the targets acted upon likely play a role in mitogenesis. These tyrosine phosphorylation events were not blocked by a selective PKC inhibitor or apparently mediated by increased intracellular calcium. Thus, since two independent pathways, the GPCR and tyrosine phosphorylation pathways, can drive the vascular smooth muscle cells into a proliferative state, it is necessary to block both independent signaling arms. This is the basis for the synergistic interaction between calcium channel antagonists and tyrosine kinase inhibitors in the surgical solution. Because the actions of the protein tyrosine kinase inhibitors in preventing vascular smooth muscle cell proliferation occur via independent molecular pathways (described above) from those involving calcium and protein kinase C, the combination of the two classes of drugs, calcium channel antagonists and protein tyrosine kinase inhibitors, is

The irrigation solution is applied to the wound or surgical site prior to the initiation of the procedure, preferably before tissue trauma, and continuously throughout the duration of the procedure, to preemptively block pain and inflammation, spasm and restenosis. As used herein throughout, the term "irrigation" is intended to mean the flushing of a wound or anatomic structure with a stream of liquid. The term "application" is intended to encompass irrigation and other methods of locally introducing the solution of the present invention, such as introducing a gellable version of the solution to the operative site, with the gelled solution then remaining at the site throughout the procedure. As used herein throughout, the term "continuously" is intended to also include situations in which there is repeated and frequent irrigation of wounds at a frequency sufficient to maintain a predetermined therapeutic local concentration of the applied agents, and applications in which there may be intermittent cessation of irrigation fluid flow necessitated by operating technique.

The concentrations listed for each of the agents within the solutions of the present invention are the concentrations of the agents delivered locally, in the absence of metabolic transformation, to the operative site in order to achieve a predetermined level of effect at the operative site. It is understood that the drug concentrations in a given solution may need to be adjusted to account for local dilution upon delivery. For example, in the cardiovascular application, if one assumes an average human coronary artery blood flow rate of 80 cc per minute and an average delivery rate for the solution of 5 cc per minute via a local delivery catheter (i.e., a blood flow-to-solution delivery ratio of 16 to 1), one would require that the drug concentrations within the solution be increased 16-fold over the desired *in vivo* drug concentrations. Solution concentrations are not adjusted to account for metabolic transformations or dilution by total body distribution because these circumstances are avoided by local delivery, as opposed to oral, intravenous, subcutaneous or intramuscular application.

Arthroscopic techniques for which the present solution may be employed include, by way of non-limiting example, partial meniscectomies and ligament reconstructions in the knee, shoulder acromioplasties, rotator cuff debridements, elbow synovectomies, and wrist and ankle arthroscopies. The irrigation solution is continuously supplied intraoperatively to the joint at a flow rate sufficient to distend the joint capsule, to remove operative debris, and to enable unobstructed intra-articular visualization.

total dosing levels approximately 200-fold less than were required via the intravenous route to obtain the same therapeutic effect. Given that only a small fraction of the drug delivered intra-articularly is absorbed by the local synovial tissue, the difference in plasma drug levels between the two routes of administration is much greater than the difference in total amitriptyline dosing levels.

Practice of the present invention should be distinguished from conventional intra-articular injections of opiates and/or local anesthetics at the completion of arthroscopic or "open" joint (e.g., knee, shoulder, etc.) procedures. The solution of the present invention is used for continuous infusion throughout the surgical procedure to provide preemptive inhibition of pain and inflammation. In contrast, the high concentrations necessary to achieve therapeutic efficacy with a constant infusion of local anesthetics, such as lidocaine (0.5-2% solutions), would result in profound systemic toxicity.

Upon completion of the procedure of the present invention, it may be desirable to inject or otherwise apply a higher concentration of the same pain and inflammation inhibitors as used in the irrigation solution at the operative site, as an alternative or supplement to opiates.

The solution of the present invention also has application in cardiovascular and general vascular diagnostic and therapeutic procedures to potentially decrease vessel wall spasm, platelet aggregation, vascular smooth muscle cell proliferation and nociceptor activation produced by vessel manipulation. Reference herein to arterial treatment is intended to encompass the treatment of venous grafts harvested and placed in the arterial system. A suitable solution for such techniques is disclosed in Example II herein below. The cardiovascular and general vascular solution preferably includes any combination, and preferably all, of the following: a 5-HT_2 receptor antagonist (Saxena, P. R., et. al., *Cardiovascular Effects of Serotonin Inhibitory Agonists and Antagonists*, J Cardiovasc Pharmacol 15 (Suppl. 7), pp. S17-S34 (1990); Douglas, 1985); a 5-HT_3 receptor antagonist to block activation of these receptors on sympathetic neurons and C-fiber nociceptive neurons in the vessel walls, which has been shown to produce brady- and tachycardia (Saxena et. al. 1990); a bradykinin$_1$ receptor antagonist; and a cyclooxygenase inhibitor to prevent production of prostaglandins at tissue injury sites and thereby decreasing pain and inflammation. In addition, the cardiovascular and general vascular solution also preferably will contain a serotonin$_{1B}$ (also known as serotonin$_{1D\beta}$) antagonist because serotonin has been shown to produce significant vascular spasm via activation of the serotonin$_{1B}$

component of severe burns. Holliman, C.J., et. al., *The Effect of Ketanserin, a Specific Serotonin Antagonist, on Burn Shock Hemodynamic Parameters in a Porcine Burn Model*, J Trauma 23, pp. 867-871 (1983). The solution disclosed in Example I for arthroscopy may also be suitably applied to a wound or burn for pain and inflammation control, and for surgical procedures such as arthroscopy. The agents of the solution of Example I may alternately be included in a paste or salve base, for application to the burn or wound.

VII. Examples

The following are several formulations in accordance with the present invention suitable for certain operative procedures followed by a summary of three clinical studies utilizing the agents of the present invention.

A. Example I

Irrigation Solution for Arthroscopy

The following composition is suitable for use in anatomic joint irrigation during arthroscopic procedures. Each drug is solubilized in a carrier fluid containing physiologic electrolytes, such as normal saline or lactated Ringer's solution, as are the remaining solutions described in subsequent examples.

Class of Agent	Drug	Concentration (Nanomolar): Therapeutic	Preferred	Most Preferred
serotonin$_2$ antagonist	amitriptyline	0.1-1,000	50-500	100
serotonin$_3$ antagonist	metoclopramide	10-10,000	200-2,000	1,000
histamine$_1$ antagonist	amitriptyline	0.1-1,000	50-500	200
serotonin$_{1A, 1B, 1D, 1F}$ agonist	sumatriptan	1-1,000	10-200	50
bradykinin$_1$ antagonist	[des-Arg^{10}] derivative of HOE 140	1-1,000	50-500	200
bradykinin$_2$ antagonist	HOE 140	1-1,000	50-500	200

C. Example III

Irrigation Solution for Urologic Procedures

The following drugs and concentration ranges in solution in a physiologic carrier fluid are suitable for use in irrigating operative sites during urologic procedures.

Class of Agent	Drug	Concentration (Nanomolar): Therapeutic	Preferred	Most Preferred
$histamine_1$ antagonist	terfenadine	0.1-1,000	50-500	200
$serotonin_3$ antagonist	metoclopramide	10-10,000	200-2,000	1,000
$bradykinin_1$ antagonist	[des-Arg^{10}] derivative of HOE 140	1-1,000	50-500	200
$bradykinin_2$ antagonist	HOE 140	1-1,000	50-500	200
cyclooxygenase inhibitor		100-10,000	500-5,000	3,000

E. Example V

Alternate Irrigation Solution for Cardiovascular and General Vascular Therapeutic and Diagnostic Procedures

The following drugs and concentration ranges in solution in a physiologic carrier fluid are preferred for use in irrigating operative sites during cardiovascular and general vascular procedures. Again, this solution is preferred relative to the solution set forth in Example II above for higher efficacy.

Class of Agent	Drug	Concentration (Nanomolar): Therapeutic	Preferred	Most Preferred
serotonin$_2$ antagonist	trazodone	0.1 - 2,000	50 - 500	200
cyclooxygenase inhibitor	ketorolac	100 - 10,000	500 - 5,000	3,000
endothelin antagonist	BQ 123	0.01 - 1,000	10 - 1,000	500
ATP-sensitive K^+ channel agonist	(-) pinacidil	1 - 10,000	100 - 1,000	500
Ca^{2+} channel antagonist	nisoldipine	1 - 10,000	100 - 1,000	500
nitric oxide donor	SIN-1	10 - 10,000	100 - 1,000	500

G. Example VII

Cardiovascular and General Vascular Anti-Restenosis Irrigation Solution

The following drugs and concentration ranges in solution in a physiologic carrier fluid are preferred for use in irrigation during cardiovascular and general vascular therapeutic and diagnostic procedures. The drugs in this preferred solution may also be added at the same concentration to the cardiovascular and general vascular irrigation solutions of Examples II and V described above or Example VIII described below for preferred anti-spasmodic, anti-restenosis, anti-pain/anti-inflammation solutions.

Class of Agent	Drug	Concentration (Nanomolar): Therapeutic	Preferred	Most Preferred
thrombin inhibitor	hirulog	0.2-20,000	2-2,000	200
glycoprotein IIb/IIIa receptor blocker	integrelin	0.1-10,000 x Kd	1-1000 x Kd	100 x Kd
PKC inhibitor	GF 109203X*	0.1-10,000	1-1,000	200
protein tyrosine kinase inhibitor	tyrphostin AG1296	10-100,000	100-20,000	10,000

* Also known as Go 6850 or Bisindoylmaleimide I (available from Warner-Lambert)

I. Example IX

Alternate Irrigation Solution for Arthroscopy, General Surgical Wounds, Burns and Oral/Dental Applications

An alternate preferred solution for use in irrigation of arthroscopic, general surgical and oral/dental applications is formulated the same as in the previously described Example IV, with the following substitution, deletion and additions at the concentrations set forth below:

1) amitriptyline is replaced by mepyramine as the H_1 antagonist;

2) the kallikrein inhibitor, aprotinin, is deleted;

3) a bradykinin$_1$ antagonist, [leu^9] [des-Arg^{10}] kalliden, is added;

4) a bradykinin$_2$ antagonist, HOE 140, is added; and

5) a T-opioid agonist, fentanyl, is added.

Class of Agent	Drug	Concentration (Nanomolar): Therapeutic	Preferred	Most Preferred
H_1 antagonist	mepyramine	0.1-1,000	5-200	100
bradykinin$_1$ antagonist	[leu^9][des-Arg^{10}] kalliden	0.1-500	10-200	100
bradykinin$_2$ antagonist	HOE 140	1-1,000	50-500	200
T-opioid agonist	fentanyl	0.1-500	10-200	100

1. Design Considerations

This study was intended to describe the time course of change in arterial lumen dimensions in one group of arteries and to evaluate the effect of histamine/serotonin receptor blockade on these changes in a second group of similar arteries. To facilitate the comparison of the two different groups, both groups were treated in an identical manner with the exception of the contents of an infusion performed during the experiment. In control animals (arteries), the infusion was normal saline (the vehicle for test solution). The histamine/serotonin receptor blockade treated arteries received saline containing the receptor antagonists at the same rate and at the same part of the protocol as control animals. Specifically, the test solution included: (a) the serotonin$_3$ antagonist metoclopramide at a concentration of 16.0 μM; (b) the serotonin$_2$ antagonist trazodone at a concentration of 1.6 μM; and (c) the histamine antagonist promethazine at concentrations of 1.0 μM, all in normal saline. Drug concentrations within the test solution were 16-fold greater than the drug concentrations delivered at the operative site due to a 16 to 1 flow rate ratio between the iliac artery (80 cc per minute) and the solution delivery catheter (5 cc per minute). This study was performed in a prospective, randomized and blinded manner. Assignment to the specific groups was random and investigators were blinded to infusion solution contents (saline alone or saline containing the histamine/serotonin receptor antagonists) until the completion of the angiographic analysis.

2. Animal Protocol

This protocol was approved by the Seattle Veteran Affairs Medical Center Committee on Animal Use and the facility is fully accredited by the American Association for Accreditation of Laboratory Animal Care. The iliac arteries of 3-4 kg male New Zealand white rabbits fed a regular rabbit chow were studied. The animals were sedated using intravenous xylazine (5 mg/kg) and ketamine (35 mg/kg) dosed to effect and a cutdown was performed in the ventral midline of the neck to isolate a carotid artery. The artery was ligated distally, an arteriotomy performed and a 5 French sheath was introduced into the descending aorta. Baseline blood pressure and heart rate were recorded and then an angiogram of the distal aorta and bilateral iliac arteries was recorded on 35 mm cine film (frame rate 15 per second) using hand injection of iopamidol 76% (Squibb Diagnostics, Princeton, NJ) into the descending aorta. For each angiogram, a calibration object was placed in the radiographic field of

were made at three sites in each iliac artery: proximal to the site of balloon dilatation, at the site of balloon dilatation and just distal to the site of balloon dilatation.

The diameter measurements were then converted to area measurements by the formula:

$$\text{Area} = (\text{Pi})(\text{Diameter}^2)/4.$$

For calculation of vasoconstriction, baseline values were used to represent the maximum area of the artery and percent vasoconstriction was calculated as: % Vasoconstriction = {(Baseline area - Later time point area)/Baseline area} x100.

4. Statistical Methods

All values are expressed as mean ± 1 standard error of the mean. The time course of vasomotor response in control arteries was assessed using one way analysis of variance with correction for repeated measures. Post hoc comparison of data between specific time points was performed using the Scheffe test. Once the time points at which significant vasoconstriction occurred had been determined in control arteries, the control and histamine/serotonin receptor antagonist treated arteries were compared at those time points where significant vasoconstriction occurred in control arteries using multiple analysis of variance with treatment group identified as an independent variable. To compensate for the absence of a single a priori stated hypothesis, a p value <0.01 was considered significant. Statistics were performed using Statistica for Windows, version 4.5, (Statsoft, Tulsa, OK).

5. Results

The time course of arterial dimension changes before and after balloon angioplasty in normal arteries receiving saline infusion was evaluated in 16 arteries from 8 animals (Table 23). Three segments of each artery were studied: the proximal segment immediately upstream from the balloon dilated segment, the balloon dilated segment and the distal segment immediately downstream from the balloon dilated segment. The proximal and distal segments demonstrated similar patterns of change in arterial dimensions: in each, there was significant change in arterial diameter when all time points were compared (proximal segment, $p=0.0002$ and distal segment, $p<0.001$, ANOVA). Post hoc testing indicated that the diameters at the immediate post angioplasty time point were significantly less than the diameters at baseline or at the 30 minute time point in each of these segments. On the other hand, the arterial

vasoconstriction are shown in FIGURE 9; the changes in the amount of vasoconstriction over time are significant (in the proximal segment, p=0.0008; in the distal segment, p=0.0001, ANOVA). Post hoc testing identifies the vasoconstriction at the immediate post angioplasty time point as significantly different from that present at the 30 minute time point (P<0.001 in both segments). In the distal segment, the immediate post angioplasty vasoconstriction was also significantly less than that at 5 minutes (p<0.01); no other differences in intra-time point comparisons were significant by post hoc testing.

The luminal changes in control arteries can be summarized as follows: 1) Vasoconstriction with loss of approximately 30% of baseline luminal area occurs in the segments of artery proximal and distal to the balloon dilated segment immediately after balloon dilatation. There are trends to smaller amounts of vasoconstriction in the proximal and distal segments before dilatation and at the 15 minute time point (approximately 7 minutes after dilatation) also but, by the 30 minute time point (approximately 22 minutes after dilatation), a trend towards vasodilatation has replaced the previous vasoconstriction; 2) In the balloon dilated segment, only minor changes in lumen dimensions are present, and, despite the use of a balloon with a significantly larger inflated diameter than was present in this segment at baseline, there was no significant increase in lumen diameter of the dilated segment. These findings lead to a conclusion that any effects of the putative histamine/serotonin treatment would only be detectable in the proximal and distal segments at the time points where vasoconstriction was present.

The histamine/serotonin receptor blockade solution was infused into 16 arteries (8 animals); angiographic data was available at all time points in 12 arteries. Heart rate and systolic blood pressure measurements were available in a subset of animals (Table 24). There were no differences in heart rate or systolic blood pressure when the two animal groups were compared within specific time points. Histamine/serotonin treated animals showed trends toward a decrease in the systolic blood pressure from baseline to 30 minutes (-14±5 mm Hg, p=0.04) and a lower heart rate (-26±10, p=0.05). Within the control animals, there was no change in heart rate or systolic blood pressure over the duration of the experiment.

L. Example XII

Amitriptyline Inhibition of 5-Hydroxytryptamine-Induced Knee Joint Plasma Extravasation - Comparison of Intra-Articular Versus Intravenous Routes of Administration

The following study was undertaken in order to compare two routes of administration of the 5-HT_2 receptor antagonist, amitriptyline: 1) continuous intra-articular infusion; versus 2) intravenous injection, in a rat knee synovial model of inflammation. The ability of amitriptyline to inhibit 5-HT-induced joint plasma extravasation by comparing both the efficacy and total drug dose of amitriptyline delivered via each route was determined.

1. Animals

Approval from the Institutional Animal Care Committee at the University of California, San Francisco was obtained for these studies. Male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA) weighing 300 - 450 g were used in these studies. Rats were housed under controlled lighting conditions (lights on 6 A.M. to 6 P.M.), with food and water available *ad libitum*.

2. Plasma Extravasation

Rats were anesthetized with sodium pentobarbital (65 mg/kg) and then given a tail vein injection of Evans Blue dye (50 mg/kg in a volume of 2.5 ml/kg), which is used as a marker for plasma protein extravasation. The knee joint capsule was exposed by excising the overlying skin, and a 30-gauge needle was inserted into the joint and used for the infusion of fluid. The infusion rate (250 µl/min) was controlled by a Sage Instruments Syringe pump (Model 341B, Orion Research Inc., Boston, MA). A 25-gauge needle was also inserted into the joint space and perfusate fluid was extracted at 250 µl/min, controlled by a Sage Instruments Syringe pump (Model 351).

The rats were randomly assigned to three groups: 1) those receiving only intra-articular (IA) 5-HT (1 µM), 2) those receiving amitriptyline intravenously (IV) (doses ranging from 0.01 to 1.0 mg/kg) followed by IA 5-HT (1 mM), and 3) those receiving amitriptyline intra-articularly (IA) (concentrations ranging from 1 to 100 nM) followed by IA 5-HT (1 µM) plus IA amitriptyline. In all groups, baseline plasma extravasation levels were obtained at the beginning of each experiment by perfusing 0.9% saline intra-articularly and collecting three perfusate samples over a

0.025 mg/kg. 5-HT-induced plasma extravasation is completely inhibited by an IV amitriptyline dose of 1 mg/kg, the plasma extravasation averaging 0.034 ± 0.010.

c. Effect of Intra-articular amitriptyline on 5-HT-Induced Plasma Extravasation

Amitriptyline administered alone in increasing concentrations intra-articularly did not affect plasma extravasation levels relative to baseline, with the plasma extravasation averaging 0.018 ± 0.002 (data not shown). Amitriptyline co-perfused in increasing concentrations with 5-HT produced a concentration-dependent decrease in 5-HT-induced plasma extravasation as shown in FIGURE 12. 5-HT-induced plasma extravasation in the presence of 3 nM IA amitriptyline was not significantly different from that produced by 5-HT alone, however, 30 nM amitriptyline co-perfused with 5-HT produced a greater than 50% inhibition, while 100 nM amitriptyline produced complete inhibition of 5-HT-induced plasma extravasation. The IC_{50} for IA amitriptyline inhibition of 5-HT-induced plasma extravasation is approximately 20 nM.

The major finding of the present study is that 5-HT (1 μM) perfused intra-articularly in the rat knee joint produces a stimulation of plasma extravasation that is approximately 8-fold above baseline levels and that either intravenous or intra-articular administration of the 5-HT_2 receptor antagonist, amitriptyline, can inhibit 5-HT-induced plasma extravation. The total dosage of administered amitriptyline, however, differs dramatically between the two methods of drug delivery. The IC_{50} for IV amitriptyline inhibition of 5-HT-induced plasma extravasation is 0.025 mg/kg, or 7.5×10^{-3} mg in a 300 g adult rat. The IC_{50} for IA amitriptyline inhibition of 5-HT-induced plasma extravasation is approximately 20 nM. Since 1 ml of this solution was delivered every five minutes for a total of 35 min during the experiment, the total dosage perfused into the knee was 7 ml, for a total dosage of 4.4×10^{-5} mg perfused into the knee. This IA amitriptyline dose is approximately 200-fold less than the IV amitriptyline dose. Furthermore, it is likely that only a small fraction of the IA perfused drug is systemically absorbed, resulting in an even greater difference in the total delivered dose of drug.

Since 5-HT may play an important role in surgical pain and inflammation, as discussed earlier, 5-HT antagonists such as amitriptyline may be beneficial if used during the perioperative period. A recent study attempted to determine the effects of oral amitriptyline on post-operative orthopedic pain (Kerrick et al., 1993). An oral dose as low as 50 mg produced undesirable central nervous system side-effects, such as a "decreased feeling of well-being". Their study, in addition, also showed that oral

neck to isolate a carotid artery. The artery was ligated distally, an arteriotomy performed and a 5 French sheath was introduced into the descending aorta and positioned at the level of the renal arteries. Baseline blood pressure and heart rate were recorded. An angiogram of the distal aorta and bilateral iliac arteries was recorded on 35 mm cine film (frame rate 15 per second) using hand injection of iopamidol 76% (Squibb Diagnostics, Princeton, NJ) into the descending aorta.

For each angiogram, a calibration object was placed in the radiographic field of view to allow for correction for magnification when diameter measurements were made. Infusion of either the above described test solution or a saline control solution was started through the side arm of the 5 French sheath (and delivered to the distal aorta) at a rate of 5 cc per minute and continued for 20 minutes. At 5 minutes into the infusion, a second angiogram was performed using the previously described technique. Then a 1.25 mm or a 1.50 mm rotational atherectomy burr (Heart Technology/Boston Scientific Inc.) was advanced to the iliac arteries. The rotational atherectomy burr was advanced three times over a guide wire in each of the iliac arteries at a rotation rate of 150,000 to 200,000 RPM. In each iliac, the rotational atherectomy burr was advanced from the distal aorta to the mid portion of the iliac artery between the first and second deep femoral branches. The rotational atherectomy burr was rapidly removed and another angiogram was recorded on cine film at a mean of 8 minutes after the infusion was begun.

The infusion was continued until the 20 minute time point, and another angiogram (the fourth) was performed. Then the infusion was stopped. A total of about 95 cc of the control or test solution had been infused. At the 30 minute time point (15 minutes after the infusion was stopped), a final angiogram was recorded as before. Blood pressure and heart rate were recorded at the 15 and 30 minute time points immediately before the angiograms. After the final angiogram, the animal was euthanized with an overdose of the anesthetic agents administered intravenously.

3. Angiographic Analysis

The angiograms were recorded on 35 mm cine film at a frame rate of 15 per second. Angiograms were reviewed in random order without knowledge of treatment assignment. For analysis, the angiograms were projected from a Vanguard projector at a distance of 5.5 feet. The entire angiogram for each animal was reviewed to identify the anatomy of the iliac arteries and to identify the sites of greatest spasm in the iliac arteries. A map of the iliac anatomy was prepared to assist in consistently

rotational atherectomy was performed with the rotating burr passing from the distal aorta to the mid-portion of the iliac artery. Thus, the proximal iliac artery segment and the segment designated as the site of maximal vasoconstriction were subjected to the rotating burr. The guide wire for the rotational atherectomy catheter passed through the distal segment, but the rotating burr of the rotational atherectomy catheter itself did not enter the distal segment.

Iliac artery diameters in saline treated arteries at the three specified segments are summarized in Table 25. In the proximal segment, there was no significant change in the diameter of the artery over the time course of the experiment ($p=0.88$, ANOVA). In the mid-iliac artery at the site of maximal vasoconstriction, there was a significant reduction in diameter with the largest reduction occurring at the 15 minute post-rotational atherectomy time point ($p<0.0001$, ANOVA comparing measurements at all 5 time points). The distal segment diameter did not significantly change over the time course of the experiment ($p=0.19$, ANOVA comparing all time points) although there was a trend towards a smaller diameter at the immediate post- and 15 minute post- rotational atherectomy time points.

Table 26

Iliac artery lumen diameters at specified time points in Test Solution treated arteries.

Segment	Baseline N=13	5 Minutes into Infusion N=10	Immediate Post RA N=13	15 Minute after RA N=13	30 Minutes after RA N=11
Proximal[1]	2.28±.06	2.07±.07	2.22±.05	2.42±.06	2.39±.08
Mid[2]	1.97±.06	1.79±.06	1.74±.04	1.95±.07	1.93±.08
Distal[3]	2.00±.06	1.92±.04	1.90±.04	2.00±.06	2.01±.07

RA=rotational atherectomy

[1] Proximal iliac artery measurement site, proximal to the first deep femoral branch

[2] Mid iliac artery at the site of maximal vasospasm

[3] Distal iliac artery measurement site, near or distal to the second deep femoral branch
Because of the different number of observations at the various time points, ANOVA was not performed to determine the statistical similarity/difference in diameters within specific segments.

The primary endpoint for this study was the comparison of the amounts of vasoconstriction in saline treated and test solution treated arteries. Vasoconstriction was based on arterial lumen areas derived from artery diameter measurements. Area values at the 5 minute, immediate post-rotational atherectomy and later time points were compared to the baseline area values to calculate the relative change in area. The results were termed "vasoconstriction" if the lumen area was smaller at the later time point than at baseline, and "vasodilatation" if the lumen area was larger at the later time point compared to the baseline area (Tables 27 and 28). To facilitate statistical analysis with the largest number of observations possible in both treatment groups, the test solution and saline treated artery data were compared at the immediate post- and at the 15 minute postrotational atherectomy time points.

In the proximal segment (FIGURE 13), there was essentially no change in lumen area with either treatment at the immediate post-rotational atherectomy time point, but there was some vasodilatation in this segment by the 15 minute post-rotational atherectomy time point. Test solution did not alter the results of rotational

Table 28

Amount of vasoconstriction (negative values) or vasodilatation (positive values) at specified time points in Test Solution treated arteries.

Segment	5 Minutes into Infusion N=10	Immediate Post RA N=13	15 Minute after RA N=13	30 Minutes after RA N=11
Proximal[1]	-17%±.5%	-4%±3%	14%±6%	7%±9%
Mid[2]	-14%±5%	-20%±5%	0.3%±7%	-5%±.5%
Distal[3]	-8%±.4%	-9%±.4%	1%±4%	3%±.6%

[1] Proximal iliac artery measurement site, proximal to the first deep femoral branch

[2] Mid iliac artery at the site of maximal vasospasm

[3] Distal iliac artery measurement site, near or distal to the second deep femoral branch

The hemodynamic response in the saline and test solution treated arteries is summarized in Table 29. Compared to saline treated animals, test solution treated animals sustained substantial hypotension and significant tachycardia during the solution infusion. By 15 minutes after completion of the infusion (or at the 30 minute postrotational atherectomy time point), test solution treated animals showed some partial, but not complete, return of blood pressure towards baseline.

While the preferred embodiment of the invention has been illustrated and described, it will be appreciated that various changes to the disclosed solutions and methods can be made therein without departing from the spirit and scope of the invention. For example, alternate pain inhibitors and anti-inflammation and anti-spasm and anti-restenosis agents may be discovered that may augment or replace the disclosed agents in accordance with the disclosure contained herein. It is therefor intended that the scope of letters patent granted hereon be limited only by the definitions of the appended claims.

9. The method of Claim 8, wherein the perioperative application of the solution comprises preprocedural, intraprocedural and postprocedural application of the solution.

10. The method of Claim 8, wherein the solution is continuously applied to the wound.

11. The method of Claim 1, wherein each of the MAPK inhibitor in the solution is delivered locally at a concentration of no greater than 100,000 nanomolar.

12. The method of Claim 3, wherein each of the additional agents in the solution is delivered locally at a concentration of no greater than 100,000 nanomolar.

13. The method of Claim 1, wherein each of the plurality of agents in the solution applied is included at a concentration that is sufficient to provide a predetermined level of pain/inflammation inhibitory effect at the wound when locally applied in the absence of metabolic transformation, and that is less than a concentration which would be required to provide the same predetermined level of inhibitory effect at the wound if applied in a manner which would entail metabolic transformation of the agents.

14. The method of Claim 3, wherein the at least one pain/inflammation inhibitory agent is selected from the group consisting of: serotonin receptor antagonists; serotonin receptor agonists; histamine receptor antagonists; bradykinin receptor antagonists; kallikrein inhibitors; tachykinin receptor antagonists including neurokinin$_1$ receptor subtype antagonists and neurokinin$_2$ receptor subtype antagonists; calcitonin gene-related peptide receptor antagonists; interleukin receptor antagonists; phospholipase inhibitors including PLA_2 isoform inhibitors and PLC_γ isoform inhibitors; cyclooxygenase inhibitors; lipooxygenase inhibitors; prostanoid receptor antagonists including eicosanoid EP-1 receptor subtype antagonists and eicosanoid EP-4 receptor subtype antagonists and thromboxane receptor subtype antagonists; leukotriene receptor antagonists including leukotriene B_4 receptor subtype antagonists and leukotriene D_4 receptor subtype antagonists; opioid receptor agonists including μ-opioid receptor subtype agonists, Λ-opioid receptor subtype agonists, and P-opioid receptor subtype agonists; purinoceptor agonists and antagonists including P_{2Y} receptor agonists and P_{2X} receptor antagonists; and ATP-sensitive potassium channel openers.

19. The solution of Claim 18, wherein the MAPK inhibitor and each of the additional agents in the solution is included at a concentration of no greater than 100,000 nanomolar, adjusted for dilution in the absence of metabolic transformation, at an intended local delivery site.

20. The solution of Claim 19, wherein each of the plurality of agents in the solution is included at a concentration that is less than a concentration which would be required to provide the same predetermined level of inhibitory effect at the wound if the solution was applied in a manner which would entail metabolic transformation of the agents.

21. The solution of Claim 18, wherein the at lease one additional pain/inflammation inhibitory agents are selected from the group consisting of: serotonin receptor antagonists; serotonin receptor agonists; histamine receptor antagonists; bradykinin receptor antagonists; kallikrein inhibitors; tachykinin receptor antagonists including neurokinin$_1$ receptor subtype antagonists and neurokinin$_2$ receptor subtype antagonists; calcitonin gene-related peptide receptor antagonists; interleukin receptor antagonists; phospholipase inhibitors including PLA_2 isoform inhibitors and PLC_γ isoform inhibitors; cyclooxygenase inhibitors; lipooxygenase inhibitors; prostanoid receptor antagonists including eicosanoid EP-1 receptor subtype antagonists and eicosanoid EP-4 receptor subtype antagonists and thromboxane receptor subtype antagonists; leukotriene receptor antagonists including leukotriene B_4 receptor subtype antagonists and leukotriene D_4 receptor subtype antagonists; opioid receptor agonists including μ-opioid receptor subtype agonists, Λ-opioid receptor subtype agonists, and P-opioid receptor subtype agonists; purinoceptor agonists and antagonists including P_{2Y} receptor agonists and P_{2X} receptor antagonists; and ATP-sensitive potassium channel openers.

22. The solution of Claim 18, wherein the additional pain/inflammation inhibitory agents are included at a concentration of: 0.1 to 10,000 nanomolar for serotonin receptor antagonists; 0.1 to 2,000 nanomolar for serotonin receptor agonists; 0.01 to 1,000 nanomolar for histamine receptor antagonists; 0.1 to 10,000 nanomolar for bradykinin receptor antagonists; 0.1 to 1,000 nanomolar for kallikrein inhibitors; 0.1 to 10,000 nanomolar for neurokinin$_1$ receptor subtype antagonists; 1.0 to 10,000 nanomolar for neurokinin$_2$ receptor subtype antagonists; 1 to 1,000 nanomolar for calcitonin gene-related peptide receptor antagonists; 1 to 1,000

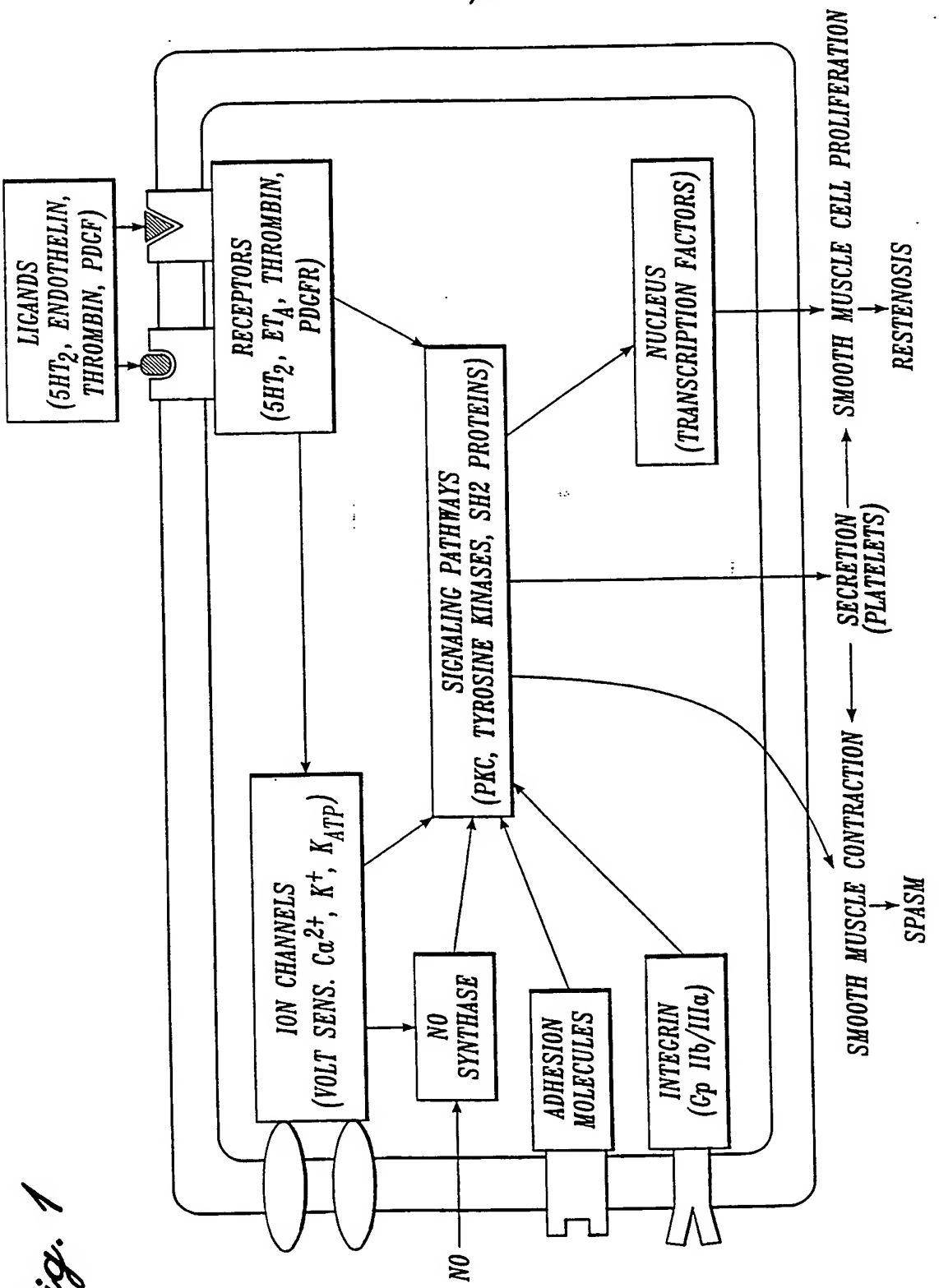


Fig. 1

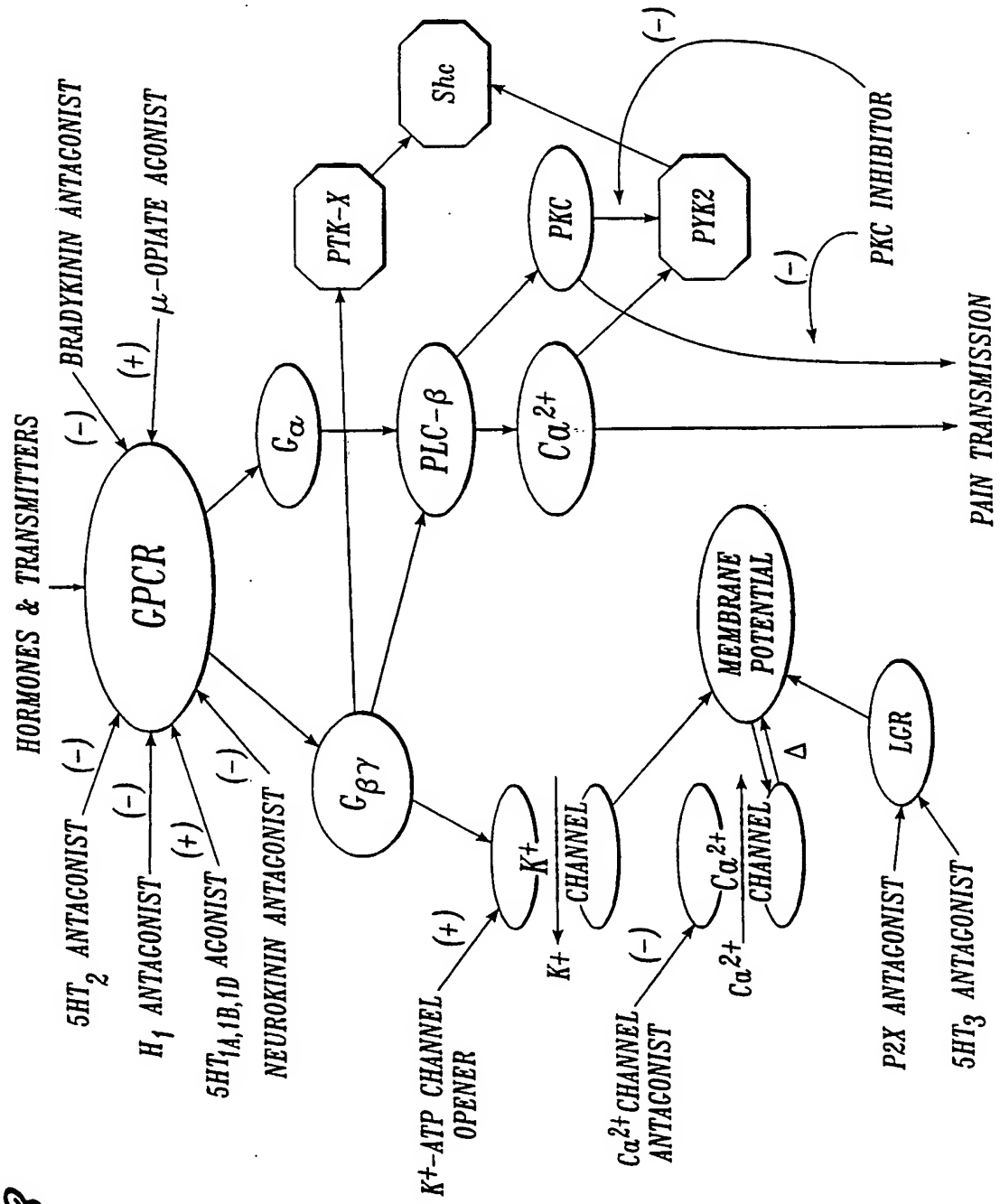


Fig. 3

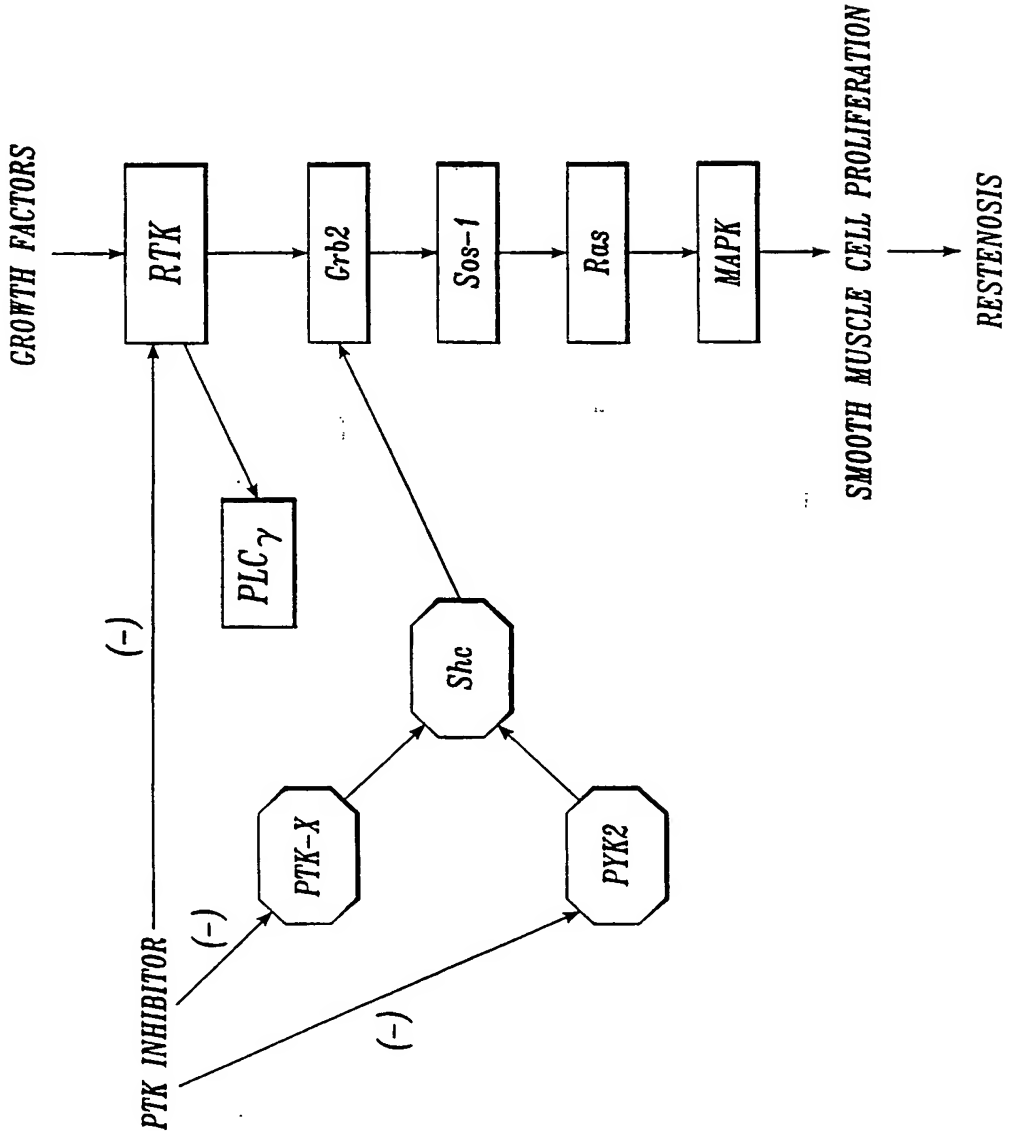


Fig. 5

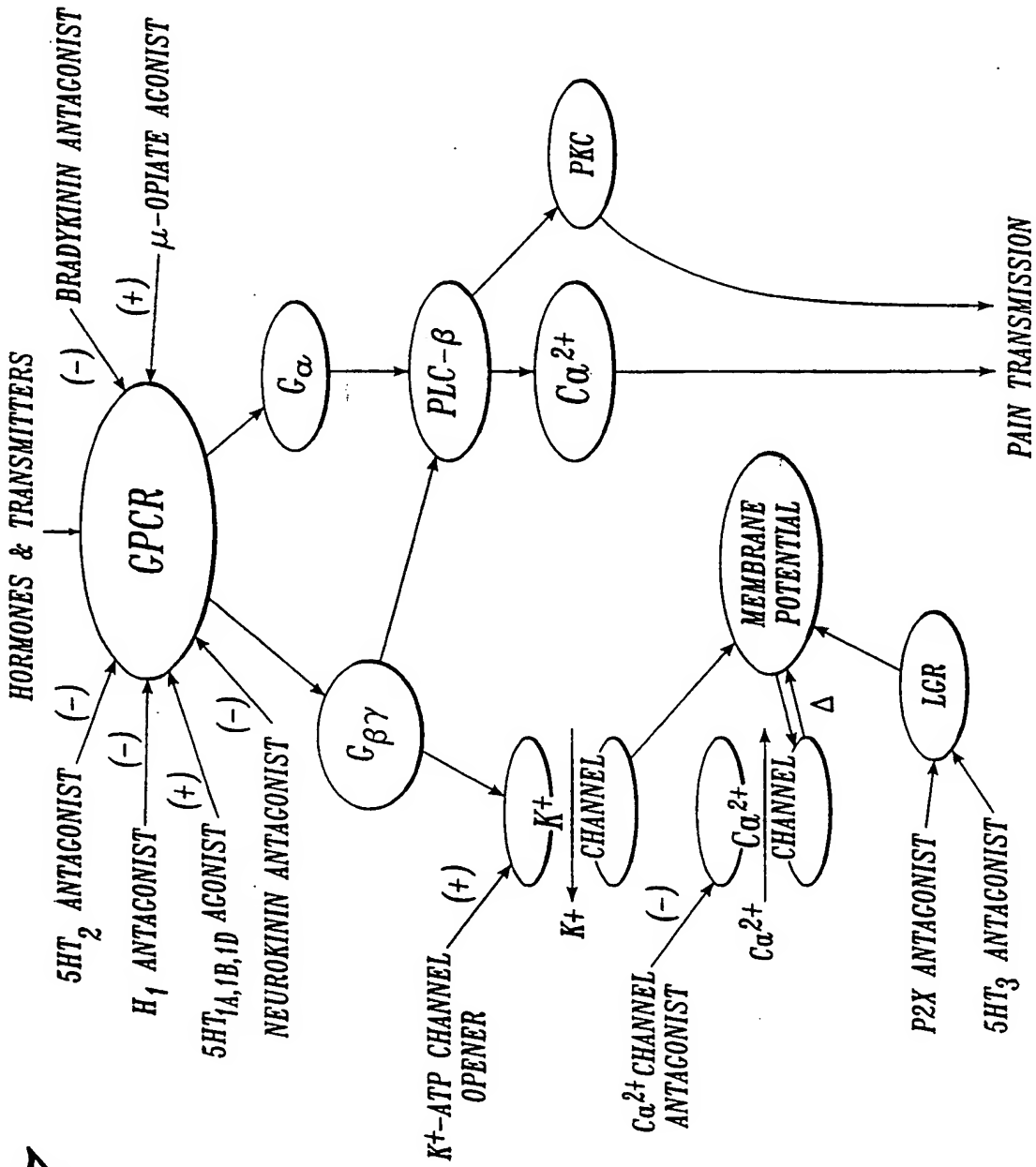


Fig. 7

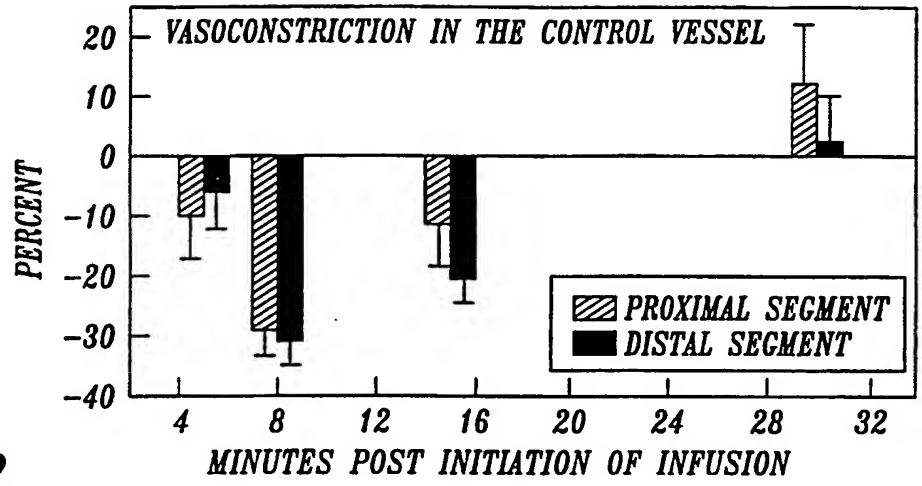


Fig. 9.

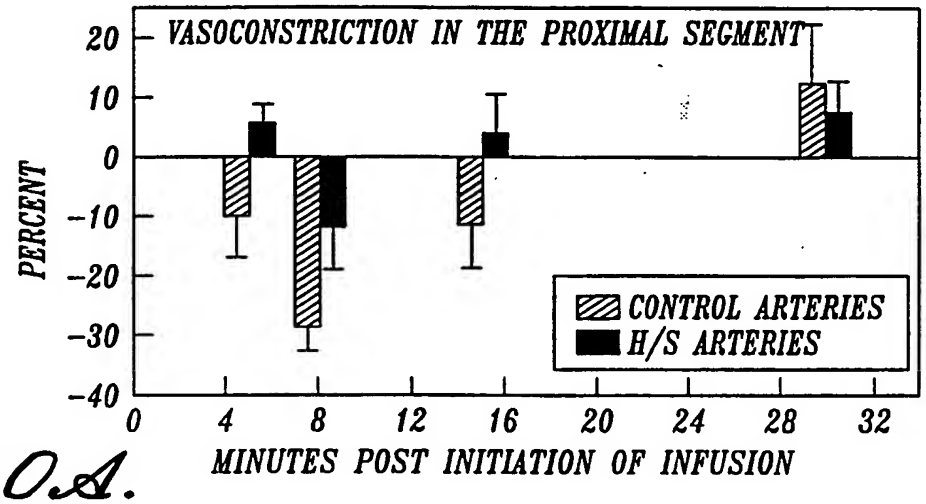


Fig. 10A.

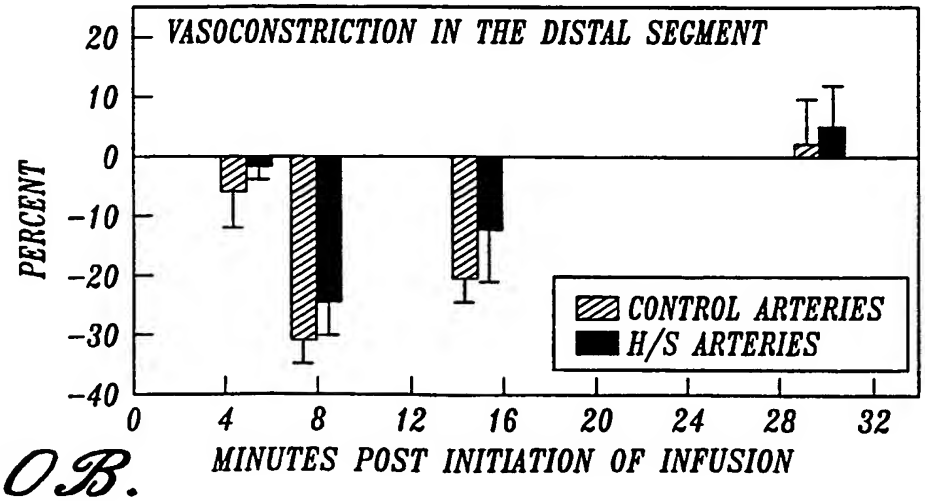
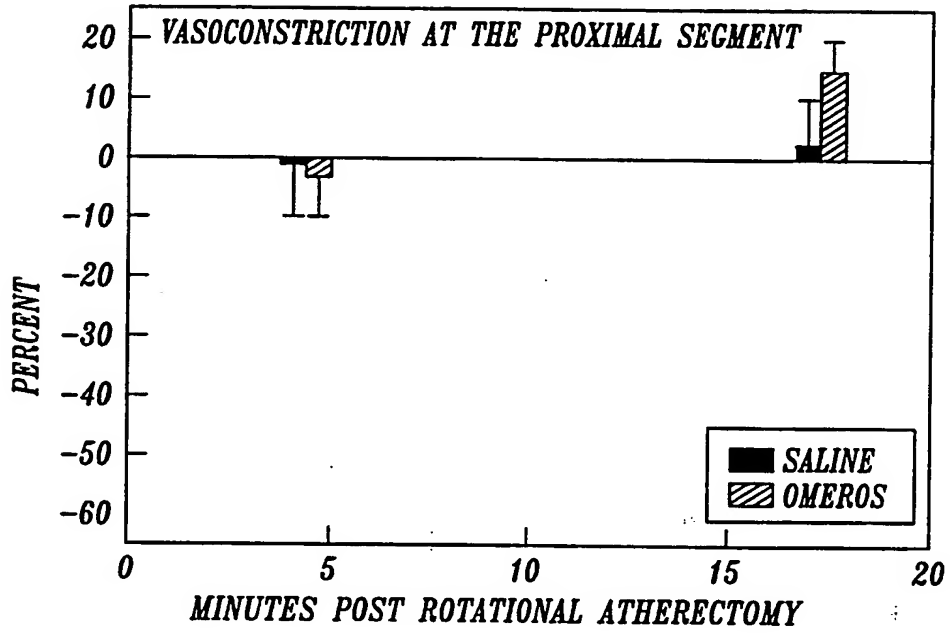


Fig. 10B.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-10
-20
-30
-40
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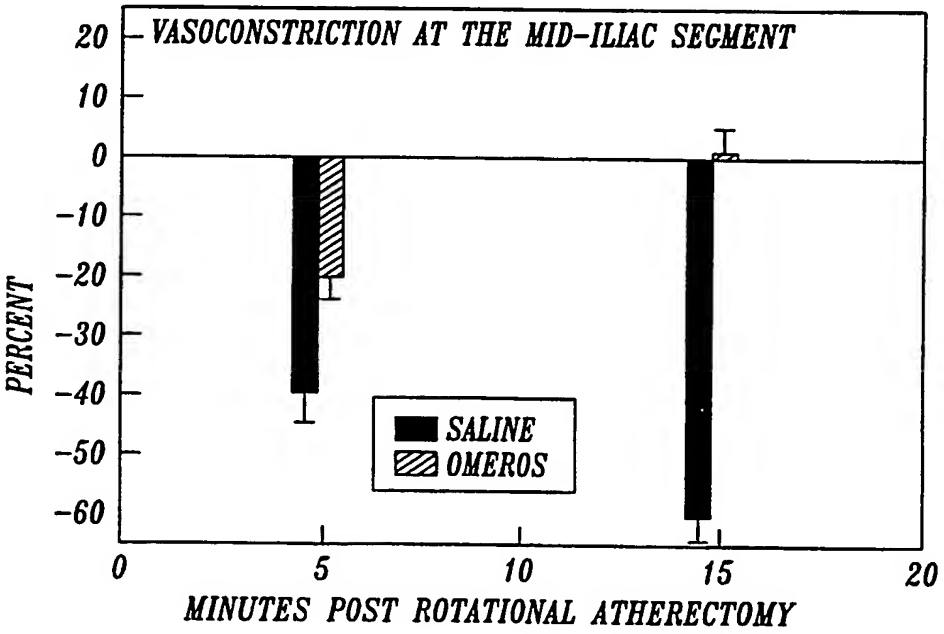
PERCENT
SALINE
OMEROS
0
5
10
15
20
MINUTES POST ROTATIONAL ATHERECTOMY

Fig. 13.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-20
-30
-40
-50
-60
PERCENT
SALINE
OMEROS
0
5
10
15
20
MINUTES POST ROTATIONAL ATHERECTOMY

Fig. 14.

INTERNATIONAL SEARCH REPORT

Int[redacted]pplication No
PCT/US 99/24625

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/352 A61K31/4439 A61P29/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 27098 A (GALULLO VINCENT P ;SALITURO FRANCESCO GERALD (US); BEMIS GUY W (US) 25 June 1998 (1998-06-25) * p.1, preamble; p.2, l.19-22; p.58, l.4-11; p.60, l.7; p.62, l.17-21; claims 25-27 and 36 *	16,18-22
Y	the whole document	1-15
X	WO 97 35855 A (SMITHKLINE BEECHAM CORP ;FEUERSTEIN GIORA Z (US)) 2 October 1997 (1997-10-02) * p.15, l.24; p.16, l.5 and l.13; p.21, bottom-p.22, l.13 *	16,17
X	US 5 525 625 A (BRIDGES ALEXANDER J ET AL) 11 June 1996 (1996-06-11) * col.4, l.4-7; col.9, l.56 *	16,17

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2000	10.03.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Uiber, P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/24625

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1(partially),3-16(partially),18-22(partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Application No
PCT/US 99/24625

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
WO 9827098	A	25-06-1998	US	5945418	A	31-08-1999
			AU	5610598	A	15-07-1998
			CZ	9902163	A	15-09-1999
			EP	0951467	A	27-10-1999
			NO	992960	A	17-08-1999
WO 9735855	A	02-10-1997	EP	0889887	A	13-01-1999
US 5525625	A	11-06-1996	AT	181913	T	15-07-1999
			AU	690400	B	23-04-1998
			AU	4245696	A	14-08-1996
			CA	2208075	A	01-08-1996
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			EP	0805807	A	12-11-1997
			JP	10512878	T	08-12-1998
			NZ	297320	A	28-05-1999
			WO	9622985	A	01-08-1996
			ZA	9600528	A	15-08-1996
WO 9721445	A	19-06-1997	AU	4467396	A	10-07-1996
			AU	6397496	A	03-07-1997
			BR	9509985	A	03-11-1998
			CA	2240256	A	19-06-1997
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			EP	0799051	A	08-10-1997
			EP	0910397	A	28-04-1999
			JP	10510540	T	13-10-1998
			NO	972687	A	07-08-1997
WO 9423039	A	13-10-1994	AU	677834	B	08-05-1997
			AU	6382394	A	24-10-1994
			CA	2157774	A	13-10-1994
			EP	0703984	A	03-04-1996
			JP	9501302	T	10-02-1997
			US	5958721	A	28-09-1999
			AU	696939	B	24-09-1998
			AU	1586195	A	29-08-1995
			CA	2182967	A	17-08-1995
			EP	0742827	A	20-11-1996
			WO	9521923	A	17-08-1995
			JP	9508795	T	09-09-1997
WO 9934792	A	15-07-1999	AU	1951499	A	26-07-1999